# The monogenic disorders of keratinization

## CLASSIFICATION AND THERAPY

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## Classification and Therapy

Een wetenschappelijke proeve op het gebied van

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The investigations described in this thesis were performed at the Department of Dermatology (head prof.dr.dr P C M van de Kerkhof) in collaboration with the Department of Pathology (head: prof.dr. D.J. Ruiter) and the Department of Human Genetics (head: prof.dr. H.H Ropers) of the University Hospital Nijmegen, The Netherlands.

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*Ter nagedachtenis van mijn vader,*

*Aan mijn moeder,*

*Aan Jeanne-Marie en Annemarijn*

CONTENTS

# General Introduction

Keratinocytes are generated in the basal layer of the epidermis and move to the surface through successive stages of differentiation This process of differentiation and cornification involves complex metabolic changes, including programmed alterations in cell proteins, membrane glycosylation and lipid biosynthesis [1 2 3] The stratum corneum is usually many cell layers thick and provides the main protective barrier of the skin and prevents 98% of the water loss The disorders of keratinization have a defect in cornification which leads to a thicker stratum corneum (hyperkeratosis) The underlying biochemical mechanisms of these disorders are believed to involve failure of desquamation (retention hyperkeratosis) or increased production of stratum corneum cells (hyperproliferation hyperkeratosis) [4] In general the disorders of keratinization are considered to be genetically determined The majority of these diseases are monogenic with a mendelian inheritance A few have polygenic inheritance or are acquired

The monogenic disorders of keratinization comprise a large and heterogeneous group of skin diseases Every year new entities are added to the list which already consists of about 70 diseases [5] Williams proposed to abandon terms like ichthyosis, palmoplantar keratoderma or erythrokeratoderma and to classify all these types of diseases as disorders of cornification by giving each entity a number [6]

Classification can be made on clinical grounds, on histological characteristics including immunohistochemical and ultrastructural findings, and by molecular biological techniques as genetic linkage studies and mutation analyses In the general introduction the different approaches will be reviewed In the appendix guidelines for classification will be presented

**Clinical manifestations**

In the monogenic disorders of keratinization hyperkeratosis can either occur isolated in the skin, or can be associated with abnormalities of other organs In the latter case the skin abnormalities mostly are the hallmark of these syndromes and multisystem birth defects The skin lesions can be present already at birth (congenital), or they can become manifest later in life (vulgar) Hyperkeratosis can occur either generalized, as in ichthyosis, or localised as in palmoplantar hyperkeratosis (Table I, appendix) Some disorders of keratinization like the epidermolytic type of epidermal naevus are manifestations of mosaicism (Table II, appendix) [7] Information about the pedigree of the patients and the clinical appearance of the hyperkeratosis can further contribute to establish the diagnosis

**Morphological identification**

Histological examination is often necessary for classification The histopathological and ultrastructural finding of epidermolytic hyperkeratosis (also called acanthokeratolysis) is typical for bullous congenital ichthyosiform erythroderma, ichthyosis bullosa of Siemens and Vorner's disease The absence of the stratum granulosum or at least the presence of

abortive keratohyalin granules is diagnostic for autosomal dominant ichthyosis vulgaris The presence of lipiddroplets in the basal keratinocytes is typical for Refsum's disease [8] Ultrastructural criteria for the classification of the heterogeneous group of lamellar ichthyosis have been developed [9-12] This classification is an important attempt to improve the nosology of lamellar ichthyosis However, the question remains to be solved whether all these morphological subtypes represent real disease entities in a genetical sense For an overview of the histopathological and ultrastructural features see Table III and IV in the appendix

**Biochemistry and cell biology**

Two-dimensional electrophoresis of proteins, derived from normal human epidermal keratinocytes, yielded 2615 cellular proteins of which 207 were identified [13] These proteins include the keratins, filaggrin, and involucrin Several attempts were made to elucidate the biochemical defect of disorders of keratinization by studying these proteins using two-dimensional gel electrophoresis, immunoblotting and immunohistochemistry Some of these proteins will now be described in detail

The keratin polypeptides are the largest family of intermediate filaments In corneocytes the keratins form 80 % of the dry cell mass More than 20 different keratins can be distinguished each coded by a different gene They are classified as Type I and Type II, the acidic and neutral-basic keratins Acidic and basic keratins form pairs as heterodimers [14-16] Figure 1 shows the structure of the two types of keratin The central rod domain is homologous for both types and contains alpha-helicoidal segments with hydrophobic amino-acids at regular distances

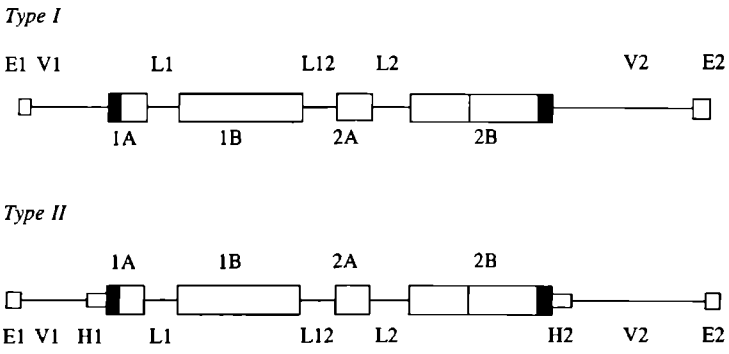


**Fig 1** Structure of the 2 types of keratin The central rod domain is homologous for both types The differences between the keratins are present in the terminal domains

The differences between the keratins are present in the terminal domains The helicoidal domains of type I and type II keratins are entwined and are stabilized by hydrophobic

interactions Two of such dimers form tetramers assembling filaments The keratinocytes of the basal epidermal layer express a dimer of the keratins 5 and 14 Whilst migrating to the suprabasal compartment, they express the new differentiation specific keratins 1 and 10 Genetically inherited structural defects in epidermal keratin filaments have been found to cause disruption of cell integrity and cytolysis in an increasing number of skin diseases including epidermolysis bullosa simplex and epidermolytic hyperkeratoses [17 20] In psoriasis and other diseases including Darier's disease loss of keratin 1 and extra bands on one-dimensional electrophoresis have been observed [21] Immunoblotting revealed the expression of a specific pair of keratins 16 and 6, 48 and 56 kD respectively, in common skin diseases such as psoriasis and epidermal carcinomas characterized by hyperproliferation [21 24] In normal skin keratin 16 and 6 are not expressed at the protein level, but they are following injury of normal skin, as the mRNAs for keratin 6 and 16 are found [25] Posttranscriptional regulation of protein expression probably permits repair following wounding In Darier's disease the keratotic lesion showed expression of these so-called hyperproliferative keratins There was also a delay in "differentiation keratin" expression in this disorder These abnormalities might be due to proteolytic damage [26] However, so far a detailed study on the profiles of keratins throughout the spectrum of disorders of keratinization is not available In view of their role in epidermal proliferation and differentiation, it is conceivable that the keratin profile might be diagnostically relevant

Retinoid treatment can cause changes in the keratin gene regulation In vitro, it inhibits keratin 1 expression and induces keratin 13 and 19 expression [27 28] So far, no data are available on the in vivo modulation of keratin expression during treatment of disorders of keratinization with retinoids Insight in the restoration of keratin expression under therapy would be relevant for the evaluation of the therapy effects

Filaggrin is an important structural protein of the corneocyt [29 33] It is synthesized as profilaggrin in the upper stratum granulosum where it is stored in the keratohyalin granules Filaggrin causes aggregation of keratin filaments into larger filaments (filaggrine) Abnormalities have been observed in restrictive dermopathy and in harlequin ichthyosis Filaggrin and profilaggrin are reduced in ichthyosis vulgaris and the reduction correlates with the severity of the disease [32] Filaggrin is increased in bullous congenital ichthyosiform erythroderma [33] The cornified envelope of the corneocyt is produced by cross linking involucrin with other membrane proteins under the influence of transglutaminase [34 38] Using antibodies against involucrin and transglutaminase, it was demonstrated that involucrin expression is altered in diseases characterized by epidermal hyperproliferation [35 38 40] Involucrin and transglutaminase are normally expressed in the upper part of the stratum spinosum and stratum granulosum In hyperproliferative states like psoriasis the number of filaggrin positive cell layers is markedly reduced and involucrin and transglutaminase are expressed prematurely in the epibasal layer [39 40]

Elias compared the stratum corneum with a brick wall in which the corneocyts are the bricks and the intercellular lipids form the mortar Normal desquamation depends on the right quality of the mortar [41] Recently the biochemical and ultrastructural aspects of

cohesion in the epidermis have been reviewed [42] During differentiation from the basal layer to the stratum corneum, lipids become less polar by the action of hydrolytic enzymes The lipids become hydrophobic, which gives the stratum corneum its water resistant quality Apart from their role to form a permeability barrier, they play a role in the dynamics of desquamation Desquamation requires breakdown of particularly cholesterol sulphate to cholesterol by steroid sulphatase to loosen cohesion between the corneocytes In X-linked recessive ichthyosis, cholesterolsulphate accumulates in the hornylayer Absence of steroidsulphatase limits intercellular lipid processing and therefore squames are retained, resulting in hyperkeratosis [43] The relevance of abnormalities in lipid metabolism to the pathogenesis of the lamellar ichthyoses is uncertain Increased n-alkanes have been reported in the stratum corneum of erythrodermic lamellar ichthyosis [44 45] These alkanes probably have an exogenous source [46] Abnormal keratinization, i c ichthyosis in conjunction with abnormal lipid metabolism, is observed in Refsum's disease, Sjogren-Larsson syndrome, neutral-lipid storage disease (Dorfman-Chanarin syndrome), and multiple steroid sulphatase deficiency syndrome [4]

Until recently the dermis was regarded as a rather inert support of the epidermis There is now more and more evidence that the dermis plays an important role in the homeostasis of the epidermis Extracellular matrix proteins, adhesion molecules such as integrins and other components, probably have a regulating function in epidermal proliferation and differentiation [47 52]

The biochemical abnormalities in disorders of keratinization are summarized in Table V of the appendix

**Treatment**

So far, therapy of disorders of keratinization has been symptomatic only In former days only treatment with keratolytics like urea, lactic acid, salicylic acid and propylene glycol were available in combination with bland emollients The therapeutic use of vitamin A in acne was already documented in 1943 by Straumfjord [53] The development of synthetic retinoids has revolutionized the treatment of disorders of keratinization [54 63] Topical all-trans-retinoic acid appeared to be effective, but due to its irritative potential its use remained restricted to the treatment of acne vulgaris [64] The availability of etretinate and later of acitretin was a major breakthrough However, the risk of systemic side-effects and in particular its potential teratogenicity limit there application Important new perspectives for the treatment of disorders of keratinization have been provided by the development of new, preferably topical, retinoids Although acitretin is a well established treatment of psoriasis, the therapeutic experience in disorders of keratinization was only fragmentary before the start of the research efforts connected with this thesis Another important development is the use of topical retinoids Retinoids belong to the ligands of the so called steroid receptor superfamily [65] The steroid receptor superfamily comprises specific receptors which bind retinoids, vitamin $D_3$, estriol, androgens and thyroxin The complex of a ligand and its receptor modulates the transcription of various genes, ultimately

yielding into modulation of epidermal proliferation and keratinization. In this respect it is of importance to evaluate ligands for other receptors of the steroid receptor superfamily in addition to the well established ligands for the retinoic acid receptor.

The following questions were addressed in this study:

(i) To what extent do clinical descriptions of phenotypes, regarding disorders of keratinization, refine and or simplify the existing nosology of monogenic disorders of keratinization (Part I)?

(ii) To what extent do immunohistochemical and molecular findings refine and or simplify the existing nosology of monogenic disorders of keratinization (Part II)?

(iii) To what extent are systemic acitretin, topical 13-cis retinoic acid, and topical vitamin $D_3$ (all ligands for members of the steroid receptor superfamily) effective in the treatment of monogenic disorders of keratinization (Part III)?

In the appendix tables are presented for an integrated diagnostic approach.

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# PART I

# Clinical Aspects

# Introduction

The monogenic disorders of keratinization comprise a large and heterogenous group of disorders which are characterized clinically by the formation of visible scales (Table I, appendix)

Classification is difficult, because of inter- and intraindividual variations, differences in nomenclature, and the large number of reported cases In recent years a number of new 'entities" have been described and manifestations which were previously considered to be distinct entities have been shown to be variants of the same disorders of keratinization

The basis of classification are the clinical findings, which provide the first indication for a possible diagnosis and the first lead to a new entity Eventually the diagnosis will be made by an integrated approach using clinical, histological and biochemical investigations In this chapter different clinical presentations of disorders of keratinization are presented Ichthyosis is a descriptive term used for a genetic disorder of keratinization showing a conspicuous scaling which is generalized and affects the whole integument The process of the disease is more or less static but may show some seasonal variation Chapter 1 describes a form of ichthyosis

Chapters 2 and 3 describe patients with a localized disorder of keratinization namely a palmoplantar hyperkeratosis The palmoplantar hyperkeratosis in itself forms a large and heterogenous group too (Table I, appendix) The cases presented here demonstrate that the group of palmoplantar hyperkeratosis is still expanding

Chapters 4, 5 and 6 show a presentation of hyperkeratosis in a naevoid or mosaic pattern The observation that CHILD-syndrome is inherited from mother to daughter provides further evidence for the concept that a X-linked dominant mutation is responsible in this disease

# Chapter 1

# Ichthyosis Bullosa of Siemens: Further Delineation of the Phenotype

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## SUMMARY

We report a third family affected with ichthyosis bullosa of Siemens, and we further delineate the clinical spectrum of this mild type of epidermolytic hyperkeratosis Erythroderma had never been present in any of the affected individuals All of them exhibited a brownish, rimpled hyperkeratosis, the main characteristic sites being the joints, the shins and the periumbilical region Blistering occurred after slight mechanical trauma and even after sweating, resulting in superficially denuded areas Two affected family members also suffered from chronic, relapsing pustular eruptions surrounded by a transient erythematous flare Light- and electron-microscopic examination revealed epidermolytic hyperkeratosis limited to the upper part of the epidermis The pustular lesions were found to be subcorneal blisters filled with neutrophils Ichthyosis bullosa of Siemens can be clearly distinguished from bullous ichthyosiform erythroderma The observation of subcorneal pustular dermatosis occurring in this phenotype provides further evidence for the genetic heterogeneity of epidermolytic hyperkeratosis

In 1937, Hermann Werner Siemens described a Dutch family with non-erythrodermic ichthyosis, which was inherited as an autosomal dominant trait [6] Six members belonging to three consecutive generations exhibited mild ichthyosis and blister formation Owing to the different distribution of the skin lesions and the absence of erythroderma, Siemens distinguished this entity from bullous congenital ichthyosiform erythroderma as described by Brocq in 1902 [3] After the original account, ichthyosis bullosa of Siemens fell into oblivion until 1986, when Traupe et al reported a second family with the disease [7] Their observations concerned eight family members over four consecutive generations The two family members examined were suffering from a mild ichthyosis with blister formation Histopathological granular degeneration (epidermolytic hyperkeratosis) limited to the upper part of the epidermis was the most prominent manifestation Taupe et al [7] argued that ichthyosis bullosa of Siemens shares the histopathological features of epidermolytic hyperkeratosis with bullous congenital ichthyosiform erythroderma, but can be distinguished from this condition by the lack of erythroderma as well as the granular degeneration being confined to the superficial layers of the epidermis

We now report on a third family with seven affected members over four consecutive generations

## CASE REPORTS

*Case 1*

The index patient ($III_7$) a 27-year-old woman, had suffered from "dry and scaly skin" since early childhood In addition to ichthyotic changes, she exhibited superficial blistering on the extensor surfaces of the arms and legs Her father, her son, and two sisters showed the same skin changes Moreover, her deceased grandmother was said to have been affected in the same manner (Figure 1) The patient also had small superficial blisters (size range, 3-5 mm) on the extensor surfaces of the arms and legs This blistering was more pronounced

during the summer and could be provoked by minor trauma or by intense sweating. Erythroderma had never been present. Except from the cosmetic point of view, the lesions did not cause complaints. The patient stated that she was the first family member to have consulted a physician. Her general health was good, and there were no signs of atopy. On physical examination, she displayed a brownish rimpled hyperkeratosis localized predominantly on the elbows, the knees and the dorsal aspects of the hands. On the shins, there were collarette-like lesions resulting from superficial blister formation (Figure 2).

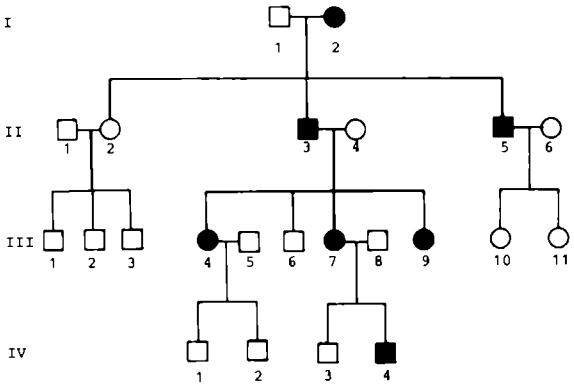



**Fig. 1.** Pedigree showing ichthyosis bullosa of Siemens occurring in four consecutive generations

*Case 2*

The 20-year-old sister ($III_9$) of the index patient was healthy except for her skin condition. Since birth, she had noticed scaling and dryness of the skin. There was no history of erythroderma. The scaling and dryness showed no seasonal variations, whereas blistering occurred more frequently in the summer. Other than these seasonal variations, her condition remained fairly stable. On physical examination, she exhibited similar skin changes that were most pronounced on the elbow, the flexural aspects of the knees, around the ankles and on the back of the hands and feet. In these regions, circumscribed, superficially denuded areas were observable. Occasionally fresh blisters ranging in size from 0.5 to 2 cm appeared.

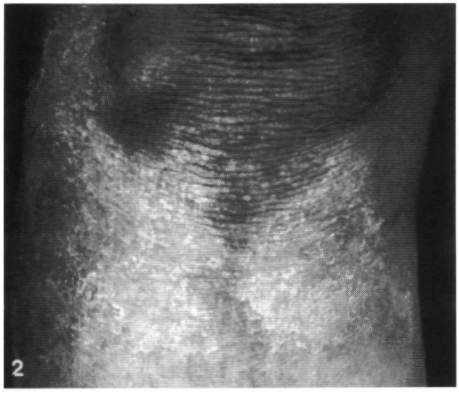
*Case 3*

The 5-year-old son ($IV_4$) of the proposita was born at term after a pregnancy without complications. Since birth, he had suffered from a generalized dryness of the skin. There were no signs of a congenital erythroderma. Blistering had started at the age of 8 month. On examination, dark-brownish, rimpled hyperkeratoses were noted, especially on the back of the hands and feet, on the flexural aspect of the knees (Figure 3) and around the ankles. Some irregularly shaped, superficially denuded lesions surrounded by a collarette-

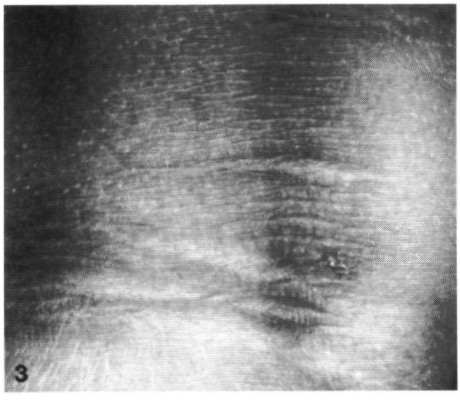
like scaling (Figure 4) were present on the dorsal aspects of the hands and on the flexural aspects of the feet, but all of these skin changes were less severe than those of his mother and his aunt.

*Case 4*

The 33-year-old sister ($III_4$) of the index patient had suffered from a scaling skin disorder since birth. There was no history of congenital erythroderma. Blistering had started 1 month after birth; it was triggered by warm, humid weather and was most pronounced on the flexural side of the knees. On examination, the patient showed the same type of skin disorder as her relatives, with rimpled brownish hyperkeratosis around the umbilicus, knees and ankles. Superficially denuded areas were observed in these areas. The hyperkeratosis was not associated with erythema.







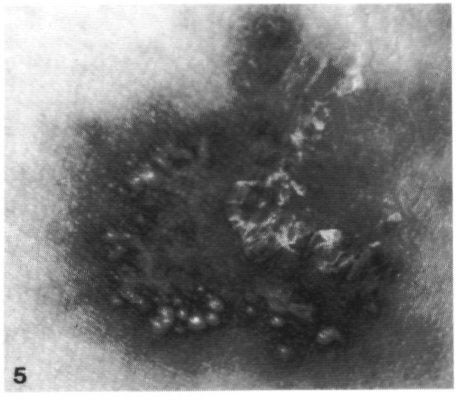




**Fig. 2.** Collarette-like lesions on the shins (case 1)

**Fig. 3.** Dark-brown, rimpled hyperkeratosis on the flexural aspect of the right knee (case 3)

**Fig. 4.** Irregularly shaped, superficially denuded lesions surrounded by collarette-like scaling (case 3)

**Fig. 5.** Groups of flaccid pustules that are gyrate in shape and surrounded by an erythematous flare (case 5)

*Case 5*

The 51-year-old father ($II_3$) of the proposita had suffered from ichthyosis since birth Blistering had started during the 1st year of life, but these episodes had become less frequent at the age of 10 years However, since the age of 15 years, he had suffered from chronic, relapsing pustular eruptions He presented with marked rimpled hyperkeratosis localized on the knees, on the elbows and around the ankles Collarette-like lesions were observed on the arms and legs In addition, flaccid, oval pustules were visible on clinically uninvolved skin localized beneath the axillary folds The pustules tended to form groups that were gyrate in shape and were surrounded, at an early stage of development, by a transient erythematous flare (Figure 5) These eruptions could be provoked by heat and were most prominent during the summer

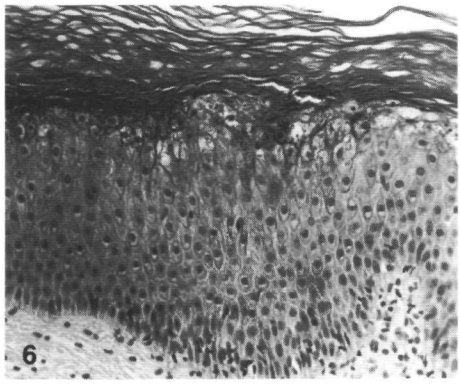
*Case 6*

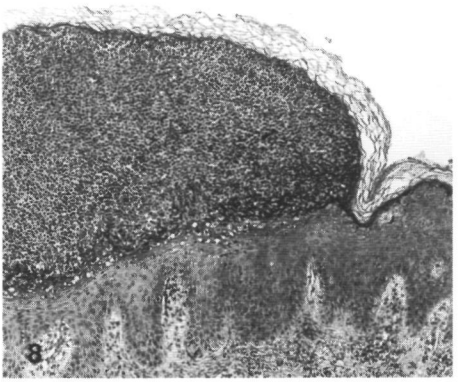
A 40-year-old uncle ($II_5$) of the index patient was suffering from non-erythematous congenital ichthyosis Blistering had started at the age of 8 month, resulting in impetigo-like lesions From the age of 10 years, the patient had been free from blistering However, like his brother, he had suffered from chronic, relapsing pustular eruptions since the age of 18 years On examination, he showed skin changes similar to those described in case 5 The collarette-like scaling, the pustular eruptions and the surrounding erythematous flare were especially marked on the trunk In his case, the pustules were present on the ichthyotic skin as well as on uninvolved skin *Histopathological findings*

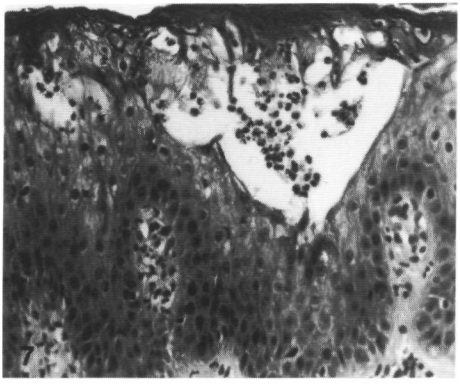
Multiple biopsies for light microscopy were taken from cases 1, 2, 5 and 6 The findings were similar for all samples In ichthyotic lesions, the epidermis was irregularly acanthotic and markedly orthohyperkeratotic PAS-positive deposits were seen within intracorneal splits, probably reflecting earlier blistering The most prominent feature was granular degeneration, or epidermolytic hyperkeratosis [1] In the stratum granulosum and the upper part of the stratum spinosum, perinuclear halos, keratohyalin clumps and hyperchromatic pycnotic nuclei were observed (Figure 6) Histopathological examination of a small, freshly induced blister in case 1 showed a more pronounced epidermolytic hyperkeratosis, resulting in blistering within the granular layer In the blister, few polymorphonuclear leukocytes were visible (Figure 7) Two biopsies of pustules taken from patients 5 and 6 revealed subcorneal blisters within an acanthotic epidermis Most of these blisters were filled with numerous polymorphonuclear leukocytes In addition, the keratinocytes of the granular layer displayed mild epidermolytic hyperkeratosis (Figure 8)

*Electron-microscopic findings*

In case 1 and 2, biopsies from ichthyotic skin lesions were taken for ultrastructural examination The cells of the granular and upper spinous layers showed marked edema, while the keratohyalin granules were fragmented Keratinocytes in the spinocellular layer displayed pronounced aggregates of tonofilaments, forming V shapes or shells around the nuclei (Figure 9)







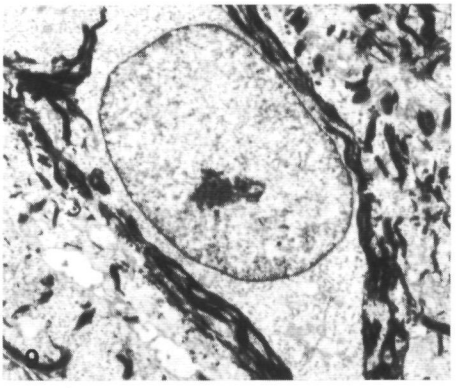


**Fig. 6.** Mild epidermolytic hyperkeratosis. H&E; x100

**Fig. 8.** Subcorneal blistering with an abundance of polymorphonuclear leukocytes. H&E; x40

**Fig. 7.** Pronounced epidermolytic hyperkeratosis with blistering within the granular layer. Note the presence of few polymorphonuclear leukocytes. H&E x100

**Fig.9.** Electron micrograph of keratinocytes in the spinocellular layer. Note the pronounced perinuclear aggregation of tonofilaments. x2600

*Treatment*

The individuals $II_3$ and $II_5$ were treated with etretinate (35 mg daily), which resulted in decreased scaling and the complete disappearance of pustular lesions. After informed consent, patient $III_4$ and $III_7$ received acitretin (35 mg daily). Within 6 weeks, there was a substantial improvement in their cutaneous condition.

## DISCUSSION

We report a third family affected with ichthyosis bullosa of Siemens. Their ichthyosis was rather mild, and the distribution of the scaly lesions in the various affected family members was constant. Their signs and symptoms strikingly resembled those described by

Siemens and Taupe et al.[6,7] Ichthyosis bullosa of Siemens does indeed appear to be a distinct entity. So far, it is not clear whether the clinical differences between ichthyosis bullosa of Siemens and bullous ichthyosiform erythroderma reflect allelism or mutations at different gene loci.

Bullous ichthyosiform erythroderma, which was first described by Brocq and was further delineated by Lapière has, up to now, been considered by most investigators to be the only form of bullous ichthyosis.[3,4,5,8] In this disease, congenital erythroderma is present at birth, and the skin is affected by inflammatory erythema and blistering. With increasing age the hyperkeratotic skin changes become more prominent, whereas the erythema subsides. In spite of light-microscopic and electron-microscopic similarities, we suggest that ichthyosis bullosa of Siemens should be distinguished from bullous ichthyosiform erythroderma on the basis of its mild clinical appearance, which is constant within a given family, as well as the absence of erythroderma.

A 15-year-old patient with epidermolytic hyperkeratosis was recently presented at the annual meeting of the British Association of Dermatologists.[2] The child had suffered from mild ichthyosis with large flaccid blister formation since the age of 3 years. Congenital erythroderma was not mentioned. There was a marked family history of epidermolytic hyperkeratosis. This family may represent a further example of ichthyosis bullosa of Siemens, suggesting that this disorder is not quite as uncommon as once supposed.

In addition to ichthyotic skin lesions, two brothers of the present family exhibited chronic, relapsing pustular eruptions that were clinically and histopathologically indistinguishable from subcorneal pustular dermatosis of the Sneddon-Wilkinson type. Some polymorphonuclear granulocytes were also observed in blisters examined in one of the patients (case 1), who did not suffer from recurrent pustular eruptions. Up to now, a familial occurrence of subcorneal pustular dermatosis has never been reported. It is rather unlikely that the occurrence of subcorneal pustular dermatosis in two brothers affected with ichthyosis bullosa of Siemens is coincidental. Rather, we would consider ichthyosis bullosa of Siemens to be a disease that either predisposes to, or mimics subcorneal pustular dermatosis. If this is so, subcorneal pustular dermatosis would be a heterogeneous phenomenon. The occurrence of subcorneal pustulosis further delineates the clinical spectrum of ichthyosis bullosa of Siemens and may help to distinguish this disease from bullous ichthyosiform erythroderma.

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# Chapter 2

# Acantholytic Ectodermal Dysplasia

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Submitted

## SUMMARY

A 33-year-old man suffered from increased vulnerability of the skin with marked plantar hyperkeratosis extending to the periungual areas. The nails were thickened because of subungual hyperkeratosis. The scalp hair was markedly curly and could easily be plucked without pain. Eyebrows and lashes as well as pubic and axillary hair were very sparse. Histopathological examination of biopsies obtained from various skin areas consistently revealed acantholysis similar toe that observed in Hailey-Hailey disease. Moreover, a scalp biopsy showed acantholysis present in the deep parts of hair follicles. Ophthalmological examination showed stellate lenticular opacities. To our knowledge this constellation of symptoms has so far not been described. We propose the designation "acantholytic ectodermal dysplasia". As the patient did not know any of his family members, no statement can so far be made regarding the mode of inheritance of this unusual trait.

## INTRODUCTION

The term "ectodermal dysplasia" is presently defined as a symptom complex of genetically determined defects involving two or more of the various ectodermal structures such as skin, hair , nails, teeth, and eyes.[1] Solomon et al.[2] proposed three criteria for the definition of ectodermal dysplasia:

(i) the trait should be congenital,

(ii) the involvement of epidermis and skin appendages should be more or less diffuse, and the same is true for mucosal or dental lesions,

(iii) the lesions are not progressive.

We here report a case presenting a constellation of ectodermal dysplastic abnormalities that to our knowledge, has not previously been described.

## CASE REPORT

A 33-year-old man suffered since birth from extreme vulnerability of the skin and recurrent cutaneous infections. Since childhood he complained of extreme plantar hyperkeratoses that hampered him when walking. (He never received oral retinoids ) In early childhood he had be placed away from a broken home, and he did not know any of this family members.

On physical examination, sharply demarcated oozing erythematous lesions were noted predominantly on the buttocks (Figure 1) and groins (Figure 2). Less severe erosions were present in other skin areas. They appeared following mild trauma and often showed secondary infection. There was, however, no definite blistering. Marked hyperkeratoses was present on the soles (Figure 3). On the toes, hyperkeratosis extended to the periungual regions (Figure 4). All of his toe nails and some of his finger nails were thickened and showed subungual hyperkeratosis (Figure 4). The palms displayed circumscribed hyperkeratotic lesions In addition, follicular hyperkeratoses were found on the thorax. The scalp hair was dark an thick and markedly curly and could easily be plucked without pain

(Figure 5). His eyebrows and lashes were markedly sparse, and his pubic and axillar hair was likewise sparse and rather loose.

Ophthalmological examination showed stellate opacities of both lenses. These cataracts were non-symptomatic. The teeth were normal and sweating was not impaired.

Histological examination of three biopsies obtained from erosive lesions of the axillar and inguinal region consistently displayed suprabasal splitting and acantholysis (Figure 6). No dyskeratosis was observed and there was no granular degeneration. Acantholysis was also observed within the hair follicles of the scalp (Figure 7).

Electron microscopical examination of a skin biopsy obtained from the elbow displayed clumping of tonofilaments and diminished desmosomal binding sites (Figure 8).

**Fig. 1.** Sharply demarcated erosive lesions on the buttocks.

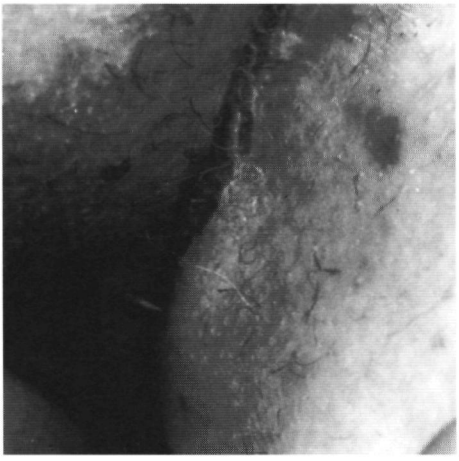

**Fig. 2.** Erosive lesions in the groins.

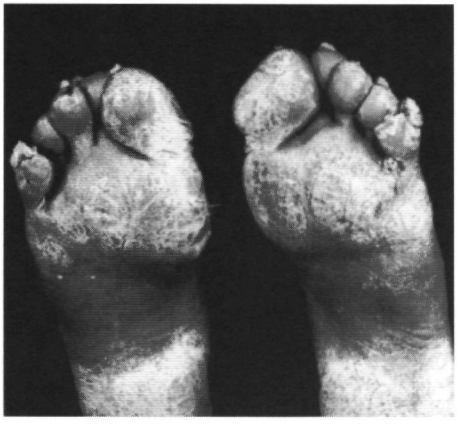

**Fig. 3.** Plantar hyperkeratosis.

**Fig. 4.** Periungual and subungual hyperkeratosis with thickening of nails.

**Fig. 5.** Curly and sparse hair

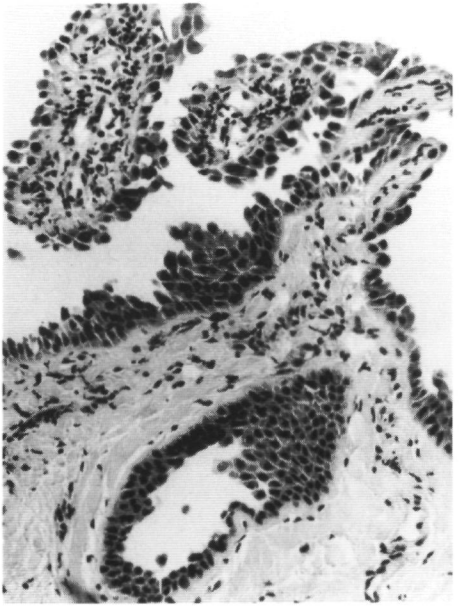

**Fig. 6.** Biopsy from an erosive lesion displaying suprabasal splitting and acantholysis (x100)

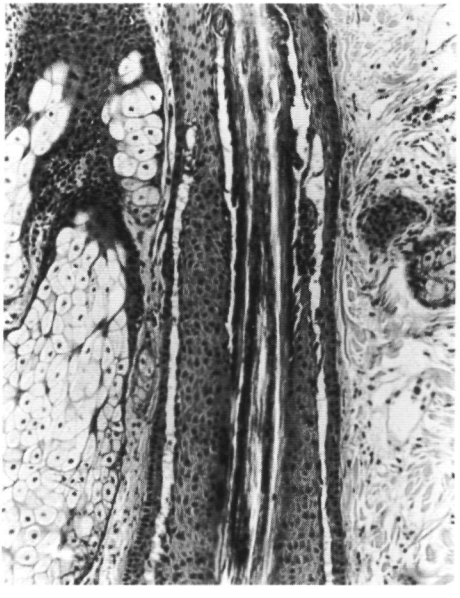

**Fig. 7.** Scalp biopsy showing acantholysis within a hair follicle. (x100)

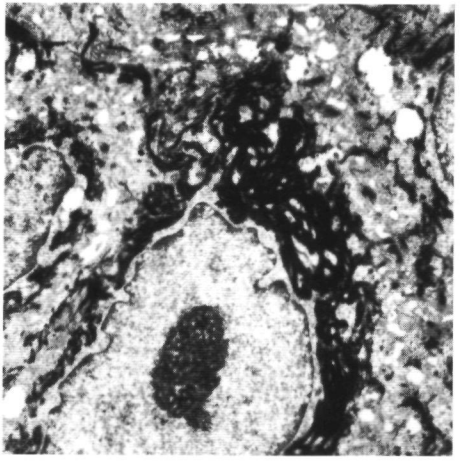

**Fig. 8.** Electron microscopical examination of a biopsy from the elbow displaying clumping of tonofilaments and diminished desmosomal binding sites. (x2600)

## DISCUSSION

This patient has a congenital disorder that diffusely affects the epidermis as well as the hair and the nails. In addition, bilateral lenticular opacities were present. Therefore, the criteria of an ectodermal dysplasia can be regarded as fulfilled.[1,2]

Thus far, no ectodermal dysplasia showing the histopathological feature of acantholysis has been described. On the other hand, this disorder can be clearly distinguished from Hailey-Hailey disease because of the associated anomalies.[3] Palmoplantar hyperkeratosis and lenticular opacities are not observed in patients with Hailey-Hailey disease. Such patients may show longitudinal leukonychia but subungual hyperkeratosis with thickening of nails is absent. However, acantholysis affecting the hair follicles was previously observed in a patient with Hailey-Hailey disease.[5] Contrasting with the present case, this patient displayed seborrhoeic dermatitis of the scalp and did not show the phenomenon of loose hair, nor palmoplantar hyperkeratosis.

This trait differs from pachyonychia congenita and ectodermal dysplasia of the Clouston type because of looseness of hair and the histopathological phenomenon of acantholysis. On the other hand, the trait differs from congenital bullous ichthyotic erythroderma of Brocq because there were no signs of epidermolytic hyperkeratosis in the various skin specimens examined. The findings obtained by electron microscopical examination excluded the various types of epidermolysis bullosa.

In our opinion the present case represents a new genodermatosis for which we propose the designation "acantholytic ectodermal dysplasia". Because the patient did not know any of his family members, we have no data to determine the mode of inheritance of this trait. We are inclined to predict, however, that future reports will show an autosomal recessive mode of transmission since otherwise this trait should occur more frequently and would therefore have been described earlier.

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# Chapter 3

# Congenital Atrichia, Palmoplantar Hyperkeratosis, Mental Retardation and Early Loss of Teeth in Four Siblings: A New Syndrome?

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## ABSTRACT

A syndrome of congenital atrichia, palmoplantar hyperkeratosis, mental retardation, and early loss of teeth was observed in four siblings (three woman and one man). The pedigree is suggestive of either an autosomal recessive mode of inheritance or the inheritance of a (small) chromosomal translocation. This combination of findings has not been previously reported and is therefore considered to be a new genetic entity.

## INTRODUCTION

In the classification of the various genetically determined forms of mental retardation, the presence of cutaneous features such as defective hair growth or palmoplantar hyperkeratosis may be useful diagnostic markers. We describe four siblings who have an unusual disorder characterized by congenital atrichia, palmoplantar hyperkeratosis, mental retardation, and early loss of teeth.

## CASE REPORTS

Four siblings had atrichia, mental retardation, and palmoplantar hyperkeratosis. They were the oldest of seven children of unrelated, healthy parents. The mother reported five spontaneous abortions (Figure 1). Of the remaining live-born children, one 39 year-old woman was alive and unaffected. Two siblings were deceased; a boy affected with trisomy 21 had died from intestinal atresia at the age of 10 days, and a woman had died at age 37 years. This woman had progressive optical atrophy, perceptual deafness, and spinocerebellar ataxia in her second decade of life. She had not displayed features such as atrichia, palmoplantar keratoderma, mental retardation, or early loss of teeth. Because an autopsy was not performed, the cause of death is unknown. Neither the children of this woman nor the children of the alive, healthy woman were affected.

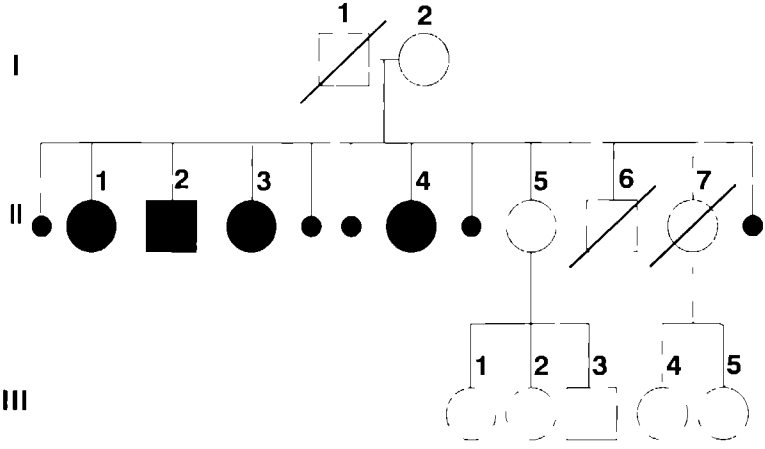


**Fig. 1.** Family pedigree

## CLINICAL FINDINGS

A 48-year-old woman (case 1), her 45-year-old brother (case 2), her 43-year-old sister (case 3) and her 42-year-old sister (case 4) had mental retardation. The retardation was severe in case 4 and moderate in the other three cases. All of the patients had alopecia since birth. At the age of approximately 1½ to 2 years, palmoplantar hyperkeratosis was noted. Teeth developed but were lost early or had to be removed because of dysplastic defects of an unknown type.

On clinical examination the scalp of each of the patients was bald (Figure 2). Patient 4, however, displayed numerous vellus hairs that were hardly visible. Hair follicle openings were preserved, and there was no evidence of scarring (Figure 3). Eyebrows and eyelashes were very sparse. Axillary and pubic hair was absent. Moreover, the patients showed an adherent hyperkeratosis on their palms and soles with spreading to the dorsal aspects and the heels (Figure 4). The hyperkeratotic lesions were surrounded by an erythematous border.

Histological examination of a scalp biopsy from patient 3 showed a normal epidermis but follicular plugging. Most follicles revealed a marked atrophy, and many contained rudimentary hair shafts. The absolute number of follicles did not appear to be reduced. Sebaceous and eccrine sweat glands were normal in size and number (Figure 5).

Microscopic examination of a biopsy specimen taken from a hyperkeratotic lesion on the sole of patient 2 showed a marked orthohyperkeratosis with focal parakeratosis. The granular layer was broadened. A marked acanthosis with elongated rete ridges was present. Serum tyrosinase measured in patient 1 and 2 was within the normal range.

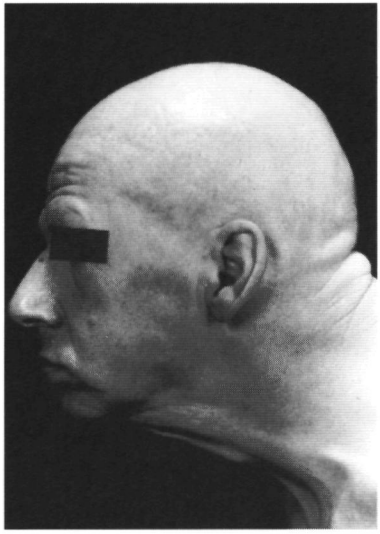

**Fig. 2.** Case 2. Atrichia of scalp and hypotrichosis of eyebrows.

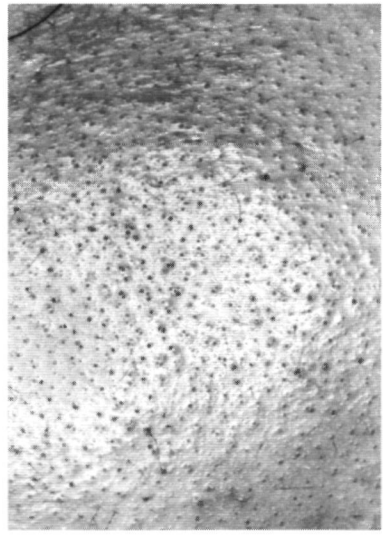

**Fig. 3.** Case 3. Presence of hair follicle openings on scalp.

**Table I. Comparison of the present syndrome to phenotypes characterized by at least two of the following features: atrichia/hypotrichosis, palmoplantar hyperkeratosis, mental retardation or dental defects.**

| | Entry in McKusick's catalogue | Presumed mode of inheritance | Atrichia (A) / Hypotrichosis (H) | Palmoplantar hyperkeratosis | Mental retardation | Dental defects | Other anomalies | References |
|---|---|---|---|---|---|---|---|---|
| Present cases | not registered | AR | A | + | + | + | Epilepsy | - |
| Alopecia congenita with keratosis palmoplantaris | 104100 | AD | A | + | - | - | Onychodystrophy and onycholysis | 2 |
| Alopecia, psychomotor epilepsy, pyorrhea and mental subnormality | 104130 | AD | H | - | + | - | Psychomotor epilepsy, pyorrhea | 3 |
| AEC syndrome | 106260 | AD | H | - | - | + | Ankyloblepharon, cleft lip and palate, hypohidrosis | 4 |
| Clouston syndrome | 129500 | AD | A/H | + | +/- | - | Onychodystrophy, hyperpigmentation | 5,6 |
| Fitzsimmons syndrome | 309560 | X-linked | - | + | + | - | Spastic paraplegia, pes cavus | 7 |
| Alopecia Contractures Dwarfism mental retardation syndrome | 203550 | AR | A | - | + | + | Growth retardation, kyphoscoliosis, dislocation of the hips, joint contractures | 8,9 |
| Alopecia-, epilepsy-, oligophrenia syndrome of Moynahan | 203600 | AR | A | - | + | - | Epilepsy | 10 |
| Alopecia - mental retardation syndrome | 203650 | AR | A | - | + | - | | 11 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| GAPO syndrome | 230740 | AR | A | - | - | + | Growth retardation, progressive optic atrophy | 12 |
| Papillon-Lefe vre syndrome | 245000 | AR | - | + | - | + | Periodontosis, gingivitis, calcification of the dura | 13,14 |
| Schopf syndrome | 245000 | AR | H | + | - | + | Multiple hidrocystadenoma of the eyelids | 15,16 |
| Richner-Hanhart syndrome | 276600 | AR | - | + | + | - | Elevation of plasma tyrosine, ocular abnormalities | 17 |
| Olmsted syndrome | not registered | AR | A/H | + | - | (+) | Periorificial hyperkeratosis, nail anomalies, joint laxity | 18,19 |
| Onycho-tricho-dysplasia with chronic neutropenia | not registered | AR | H | - | + | - | Koilonychia, onychorrhexis, recurrent infections, chronic neutropenia | 20 |
| Alopecia, oncho-dysplasia, hypohidrosis, deafness | not registered | AR | A | + | - | - | Hypohidrosis, deafness, photophobia, facial dysmorphia, short stature | 21 |
| Congenital atrichia, nail dystrophy, abnormal facies and retarded psychomotor development | not registered | AR | A | - | + | - | Onychodystrophy | 22 |
| Hereditary palmoplantar hyperkeratosis, congenital alopecia, onycho-dystrophy, enamel dysplasia | not registered | sporadic case | H | + | - | + | Koilonychia | 23 |

AR, autosomal recessive, A, atrichia, ACD, alopecia, contractures and dwarfism, AD, autosomal dominant, H,hypotrichosis, AEC ankyloblepharon, ectodermal defects, and cleft lip/palate, GAPO, growth retardation, alopecia, pseudo-anodontia, and optic atrophy

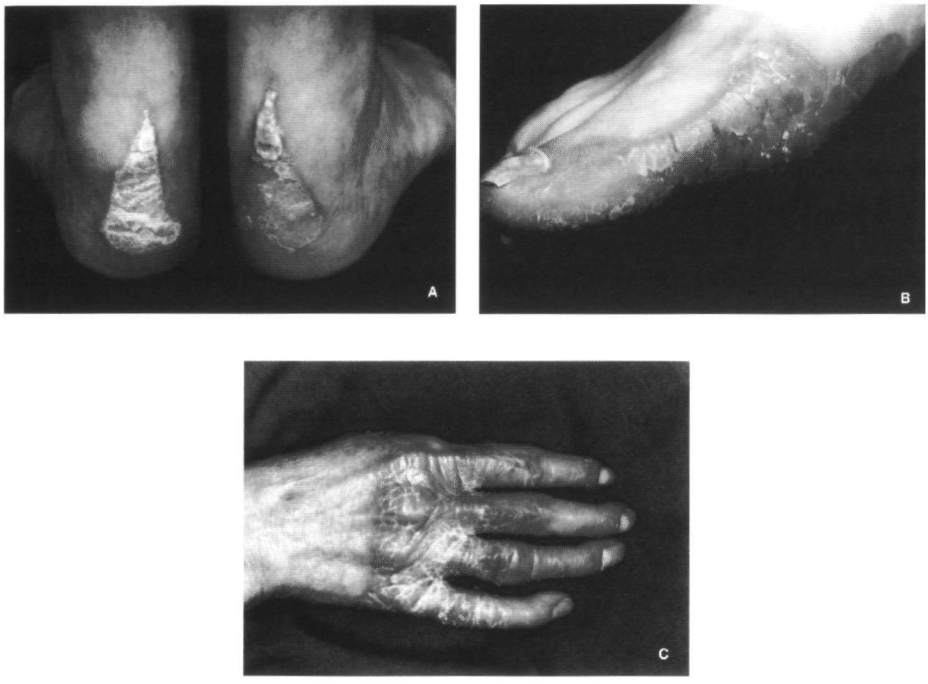


**Fig. 4. a)** Case 1. Adherent hyperkeratosis on the heels. **b)** Case 2. Plantar hyperkeratosis extending to dorsal aspect of foot. **c)** Case 4. Palmar hyperkeratosis extending to dorsal aspect of hand.

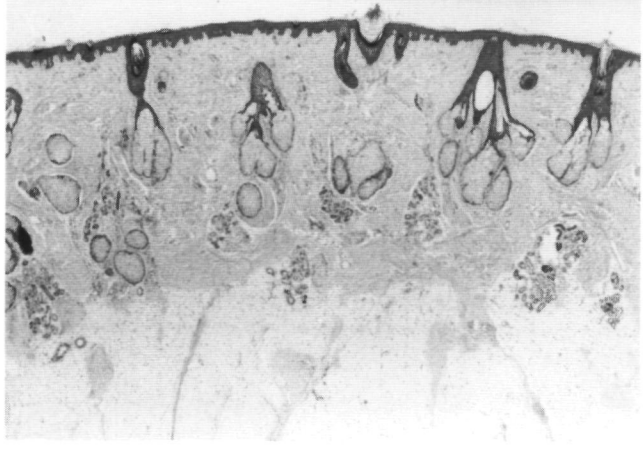

**Fig. 5.** Scalp biopsy specimen obtained from case 3 shows follicular plugging and atrophic hair follicles containing rudimentary hair shafts.

## CYTOGENETIC STUDIES

To exclude any chromosomal abnormality, routine cytogenetic studies were performed on G-banded chromosomes from cultured peripheral lymphocytes of patients 2 and 4 These studies a revealed a normal male and female karyotype, respectively. Because several spontaneous abortions, four identical patients, and three unaffected individuals were present in a single generation and both parents were healthy, the pedigree was suspect for the inheritance of a (small) chromosomal translocation. Therefore, the chromosomes of the mother were studied by means of high-resolution banding technique, but, again, no aberrations were found.

## DISCUSSION

The main features observed in these patients were congenital atrichia, palmoplantar hyperkeratosis, mental retardation, and early loss of teeth. Some phenotypical variation was noted. in patient 4 the scalp showed numerous vellus hairs, whereas the other patients were completely bald. Patient 4 was severely retarded, necessitating institutionalization. Palmoplantar hyperkeratosis and dental defects were consistent features in all four patients. Patient 2 also had epilepsy, but this finding was absent in the other siblings and therefore may be unrelated to this hereditary syndrome.

For differential diagnosis we considered 17 syndromes characterized by at least two of the four main symptoms observed in the present family (Table I). These syndromes included four autosomal dominant, one X-linked, and eleven autosomal recessive disorders and one sporadic phenotype. None of these phenotypes corresponded to the syndrome described here.

The pedigree is suggestive for an autosomal recessive mode of inheritance. However, the presence of a very small unbalanced chromosomal abnormality in the patients as a result of a balanced translocation in one of the parents is also a possibility that is not fully excluded by our cytogenetic findings. We suggested that this syndrome be considered a new entity within the heterogenous group of ectodermal dysplasias.

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# Chapter 4

# Naevus Corniculatus: A New Acantholytic Disorder

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G Kolde

Br J Dermatol 1990; 122: 107-112

## SUMMARY

We describe a 33-year-old man with an unusual epidermal naevus that followed the lines of Blaschko There were filiform hyperkeratoses, large cutaneous horns and lesions that resembled giant comedones and linear hyperkeratotic plaques All of these lesions showed acantholysis without dyskeratosis As the disorder is characterized by multiple small or large horn-like processes, we propose the term 'naevus corniculatus'

Various types of epidermal naevi have been described, such as the common verrucous type, the epidermolytic type[1], and the inflammatory linear verrucous epidermal naevus (ILVEN) [2] These are not simply variants of each other, but are regarded as being distinct entities and all of them have a linear arrangement that follows the lines of Blaschko [3]
We report an epidermal naevus that differs from the types known so far, and for which we propose the term 'naevus corniculatus'

## CASE REPORT

A 33-year-old patient had a hyperkeratotic skin disorder from birth The family history was non-contributory and the patient had a daughter who was normal Physical examination revealed an epidermal naevus that was arranged in streaks and involved the entire body with the exception of the head (Figure 1) The distribution of the skin lesions followed the lines of Blaschko Most of the individual lesions consisted of horn-like processes (Figure 2) and some areas were covered with filiform hyperkeratoses (Figure 3) Other areas showed large cutaneous horns, measuring up to 2 cm The patient was able to remove these horns without any pain, to leave a cavity lined by an erythematous keratotic wall In other areas, the hyperkeratoses resembled giant comedones Some of the lesions coalesced to form large linear verrucous plaques which were especially thick on the soles and were the cause of pain whilst walking In 1970 some of the large hyperkeratotic lesions of the right sole had been removed surgically, with subsequent grafting of skin obtained from the right thigh

*Light and electron microscopical findings*

During the past 20 years, eight biopsies were taken of lesions from various sites Light microscopy of these biopsies showed a thickened epidermis with marked hyperorthokeratosis (Figure 4) The most prominent feature, however, was acantholysis with clefting both suprabasally and in the upper epidermis, resulting in the appearance of a 'dilapidated brick wall' (Figure 5) Dyskeratotic cells were only rarely noted
On electron microscopy the keratinocytes showed thickened tonofilament bundles as well as elongated and branching microvilli (Figure 6 a-c) The wavy tonofilament aggregates surrounded the nuclei in a whorling configuration The microvillous projections only rarely contained tonofilaments and desmosomal attachments These changes were present in the suprabasal layer and were more prominent in the upper epidermis Widening of the intercellular spaces, resulting in blister formation, was found throughout the entire

epidermis. There was no dyskeratotic degeneration of the acantholytic cells. The granular layer showed a regular deposition of keratohyalin granules on the thickened tonofilaments. The horny layer was markedly thickened but did not show pathological inclusions.

**Fig. 1.** Naevus corniculatus, arranged in a systemized pattern following the lines of Blaschko

**Fig. 2** Linear arrangement of horn-like processes on the left hand.

**Fig. 3** Filiform hyperkeratoses on the trunk.

**Fig. 4** Cutaneous horn showing cleavage within the underlying epidermis (original x30).

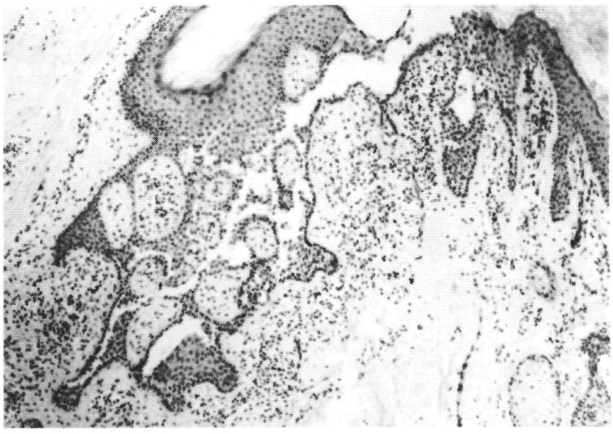

**Fig. 5** Intraepidermal cleavage with partial adhesion of the acantholytic prickle cells, giving the impression of a dilapidated brick wall (original x80)

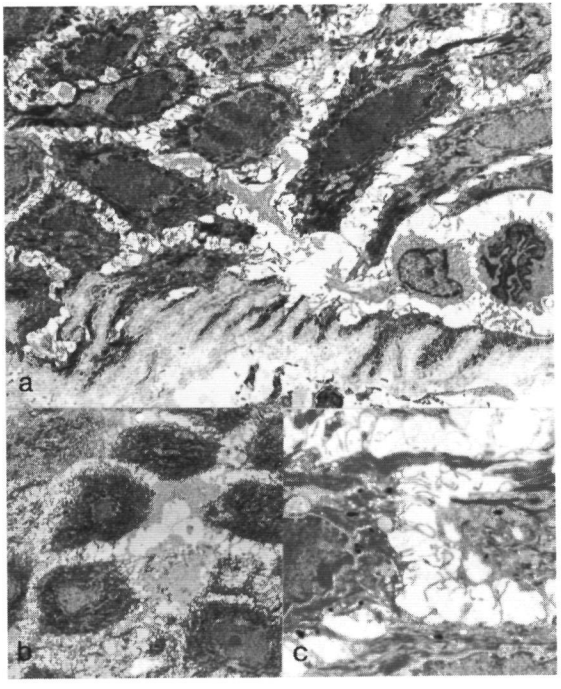


**Fig. 6 a.** Low-power electron microphotograph of the basal and suprabasal epidermis showing initial acantholysis with widened intercellular spaces (x2800) **b.** Acantholytic cells within the prickle cell layer showing characteristic disorganisation of tonofilament bundles (x1400) **c.** High power electron microphotograph of the atypical microvillous projections of keratinocytes. There are numerous elongated microvilli without tonofilament-desmosome complexes (x6500)

## DISCUSSION

This uncommon type of epidermal naevus was characterised by acantholysis and we were not able to find a similar case in the available literature. Clearly this naevus is neither Hailey-Hailey disease[4], Darier's disease[5] nor familial dyskeratotic comedones.[6] The systematized linear distribution is similar to that observed in acantholytic dyskeratotic epidermal naevus.[7,8] In this condition, however, the individual lesions resemble those of Darier's disease[9,10] and there are neither filiform horns nor comedo-like lesions as observed in our patient. The light and electron microscopical findings of our case resembled those seen in Hailey-Hailey disease[11] rather than Darier's disease.[5] Our case is also fundamentally different from the relapsing linear acantholytic dermatosis as described by Vakilzadeh and Kolde[12], the individual lesions being persistent and inflammation only rarely occurring.

In conclusion, this naevus seems to be a new skin disorder Its clinical, histopathological and ultrastructural features separate it from any other genetic acantholytic skin disorders and, in particular, from other linear conditions such as relapsing linear acantholytic dermatosis and acantholytic dyskeratotic epidermal naevus.

As horn-like processes are the clinical hallmark of the disorder, we propose the term 'naevus corniculatus'. The word corniculatus means 'covered with small horn-like processes or horns'.[13] The fact that the cutaneous horns can reach such a considerable size despite acantholysis at their base, may be explained by the almost complete absence of inflammation. This contrasts with that seen in other acantholytic skin disorders though the phenomenon of acantholysis may explain why many of these horns can easily be removed without pain or bleeding.

It is likely that this naevus originated from a somatic mutation occurring at an early stage of embryogenesis, or from a gametic half chromatid mutation [14] Whether the distribution is bilateral or unilateral, and whether the involvement is widespread or localized, will depend on the time during embryonic development when the somatic mutation occurred.

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# Chapter 5

# CHILD-Syndrom bei Mutter und Tochter

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**ZUSAMMENFASSUNG**

Ein 15jahriges Madchen mit der typischen Symptomatik des CHILD-Syndroms (kongenitale Hemidysplasie mit ichthyosiformem Navus und Gliedmaßendefekten) wird beschrieben Als begleitende ipsilaterale Anomalien wurden systematisierte Hypotrichosis, Blockwirbel, Halbwirbel, Hypoplasie der Beckenschaufel und Agenesie der Niere beobachtet Bei der gezielten Befragung der Mutter ergab sich, daß in ihrer Jugend lineare ichthyosiforme Hautveranderungen an 3 verschiedenen Korperstellen bestanden hatten Diese hatten sich im fruhen Erwachsenenalter spontan zuruckgebildet und dabei streifenformige Kahlstellen sowie eine Onychodystrophie an einem Finger hinterlassen Damit ist zum ersten Mal die Vererbung dieser Genodermatose von der Mutter auf die Tochter nachgewiesen Das postulierte Konzept der X-chromosomal-dominanten Vererbung mit Letalwirkung für mannliche Embryonen wird hierdurch untermauert Wir ziehen den Schluß, daß die Mutter eines Madchens mit CHILD-Syndrom so lange nicht als phanotypisch gesund betrachtet werden kann, als sie nicht sorgfaltig auf Minimalsymptome der Haut oder des Skelettes untersucht worden ist

**SUMMARY**

A 15-year-old girl with the typical signs and symptoms of the CHILD syndrome (congenital hemidysplasia with ichthyosiform nevus and limb defects) is described Associated ipsilateral anomalies included systematized hypotrichosis, fused vertebrae, hemivertebrae, pelvic hypoplasia and renal agenesis During a careful inquiry, her mother reported that during her own adolescence she had linear ichthyosiform skin changes localized in 3 different regions of her body These plaques had disappeared spontaneously in early adulthood, leaving permanent lesions in the form of hairless streaks and a dystrophic fingernail Hence this is the first report of a mother-to-daughter transmission of this genodermatosis, lending further support to the proposed concept of X-linked dominant inheritance with lethality for male embryos We conclude that the mother of a girl suffering from the CHILD syndrome cannot be considered to be unaffected unless a meticulous examination of her skin and bones has ruled out even minimal signs of involvement

Das CHILD-Syndrom gehort zur heterogenen Gruppe der Epidermalnavus-Syndrome Ursprunglich galt CHILD als Abkurzung fur "congenital hemidysplasia with ichthyosiform erythroderma and limb defects" [8] Da die ichthyosiformen Hautveranderungen jedoch von sehr geringer Ausdehnung sein konnen und alle Merkmale eines Navus aufweisen, erscheint folgende Interpretation des Akronymus CHILD sinnvoller "Congenital hemidysplasia with ichthyosiform *nevus* and limb defects" [6]

Wahrend andere Epidermalnavus-Syndrome wie das Schimmelpenning-Feuerstein-Mims-Syndrom und das Proteus-Syndrom grundsatzlich sporadisch auftreten[5], besteht beim CHILD-Syndrom die Besonderheit, daß er erblich ist Im jahre 1980 habben wir postuliert, daß diesem Phanotyp ein X-chromosomal-dominantes Gen mit Letalwirkung fur mannliche

Embryonen zugrundeliegt[8] Dieser Erbmodus wurde primar nicht aus Familienbeobachtungen abgeleitet, sondern aus dem Uberwiegen des weiblichen Geschlechts im Verhaltnis 19 1 und aus der asymmetrischen, mosaikartigen Verteilung der Defekte, die an ein X-Inaktivierungsmuster denken ließ

Das Konzept ist in der humangenetischen Literatur mit Zustimmung aufgenommen worden [13 21] Es bedarf jedoch noch der Untermauerung durch Familienbeobachtungen Aus diesem Grunde halten wir die folgende Kasuistik für mitteilenswert

## KASUISTIK

Ein 15 jahriges Madchen aus Jugoslawien wurde in der Hautklinik der Universitat Nijmegen vorgestellt wegen eines Fehlbildungssyndroms, für das bisher keine befriedigende Diagnose gefunden worden war Bei der Untersuchung stellte sich heraus, daß die Mutter denselben Phanotyp in schwacher Ausprägung aufwies

*Patientin 1 (Tochter)*

Bei der Geburt des Madchens waren die Mutter und der Vater beide 29 Jahre alt Es handelte sich um die erste und einzige Schwangerschaft Ab dem 3 Schwangerschaftsmonat mußte die Mutter Bettruhe halten wegen eines Abortus imminens, Sie bekam Hormone, Vitamine und milde Sedativa Gleich nach der Geburt fiel eine Hypoplasie der rechten Korperhalfte mit Asymmetrie des Gesichtes und Verkurzung der Extremitaten auf Bei der stationaren Untersuchung in der Kinderklinik der Universitat Zagreb zeigte das Pneumenzephalogramm eine symmetrische Erweiterung der lateralen Ventrikel Im Ausscheidungsurogramm war auf der rechten Seite keine Nierenfunktion erkennbar, und sowohl szintigrafisch als auch sonografisch wurde nachgewiesen, daß die rechte Niere fehlte Bei der Rontgenuntersuchung des Skelettes wurden außer einer asymmetrischen Verkurzung der langen Rohrenknochen auch unregelmaßig verkurzte Phalangen an der rechten Hand und am rechten Fuß gefunden Das Os cuboideum fehlte auf der rechten Seite Als das Kind 3 Wochen alt war, entstanden scharf umschriebene gerotete Areale mit gelblicher Schuppung auf der rechten Korperseite an Vulva, Perineum und Oberschenkel und einige Monate spater auch am rechten Fuß Die Diagnose der Kinderklinik Zagreb lautete "Russell-Silver-Syndrom, Agenesie der rechten Niere, Dermatitis desquamativa Leiner"

Im 3 Lebensjahr wurde an der rechten Halsseite eine branchiogene Zyste entfernt Im 5 Lebensjahr entstanden ichtyosiforme entzundliche Plaques in der rechten Achsel und am linken Vorfuß Ab dem 12 Lebensjahr entwickelten sich weitere navoide ichthyosiforme Hautveranderungen v a auf der rechten Korperhalfte (Nacken, Perioralregion, submammare Region, Ellenbeuge, Kniekehle, Hand), aber auch auf der linken Seite (Perioralregion, Ellenbeuge, Daumen)

Im 12 Lebensjahr erfolgte eine weitere stationare Untersuchung in der Kinderklinik der Universitat Zagreb Die Rontgenuntersuchung zeigte eine linkskonvexe Skoliose und leichte Kyphose, Halbwirbel (T8, T11, L1), Blockwirbel (T10/T11), eine Asymmetrie des

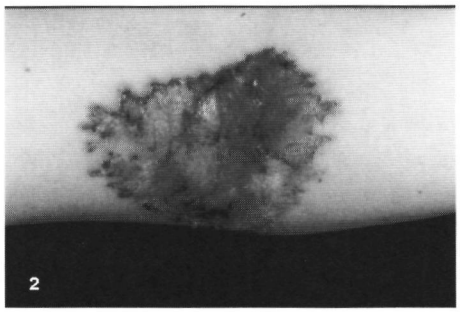
Beckens und eine Agenesie der mittleren Phalanx aller Zehen rechts Bei Intelligenzprufungen lagen die Ergebnisse leicht uber dem Durchschnitt Aufgrund der Untersuchung von Semidunnschnitten lautete die Stellungnahme des Dermatohistopathologen (Dr Dobric) "Es bestehen nicht genugend Hinweise fur Psoriasis, denn die Veranderungen sind nahezu ausschließlich auf die Epidermis beschrankt Die Hautveranderungen sollten im Zusammenhang mit dem Syndrom gesehen und klinisch diagnostiziert werden " Die Entlassungsdiagnose lautete "Russell-Silver-Syndrom und Dermatitis psoriasiformis"
Im 13 Lebensjahr ist an der rechten Hand eine der navoiden Hautveranderungen exzidiert worden, ohne daß ein Rezidiv aufgetreten ist

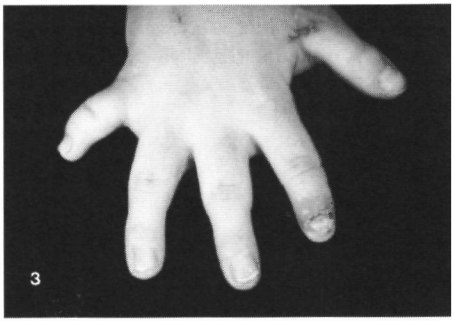
*Jetziger Befund*
Das 15jahrige Madchen macht einen aufgeweckten Eindruck und weiß sich in englischer Sprache differenziert auszudrucken Auch die Geschlechtsentwicklung ist altersentsprechend Es besteht eine ausgepragte Dysplasie der rechten Korperhalfte mit starker Verkurzung der Gliedmaßen, leichter Asymmetrie des Gesichtes und multiplen scharf umschriebenen, geroteten Hautarealen, die von gelblichen wachsartigen Schuppen bedeckt sind, teilweise aber auch nassen und Schuppenkrusten aufweisen (Abb 1 und 2) Auf der rechten Seite sind befallen Perioralregion, Nacken und Hinterhaupt, Beugeseiten der großen Gelenke, submammare Region Vulva und Rima ani sowie Hand und Fuß (Abb 3 und 4) Es besteht eine ausgepragte Dysplasie der Nagel Am rechten Fuß weisen die Interdigitalraume papillomatose, nassende Proliferationen auf In geringerer Auspragung bestehen entsprechende Hautveranderungen auch auf der linken Korperhalfte in der Ellenbeuge sowie an der Hand und am Vorfuß Auf der rechten Seite fehlt an umschriebenen Stellen die Behaarung nicht nur im Bereich des ichthyosiformen Navus (z B Hinterhaupt), sondern auch dort, wo die Haut im ubrigen normal aussieht (z B Mons pubis und Fußrucken) (Abb 1 und 4) Am linken Arm besteht eine systematisierte Haarlosigkeit in Streifenform, entsprechend dem Muster der Blaschko-Linien

*Histologische Untersuchung*
Uber einer akanthotischen Epidermis sieht man eine ausgepragte Hyperkeratose, uberwiegend vom Typ der Parakeratose Die Breite des Stratum ganulosum wechselt zwischen 2 und 6 Zellagen Im oberen Korium besteht ein lumphozytares Infiltrat, durchmischt mit eosiophillen Granulozyten und Plasmazellen

*Therapie und Verlauf*
Zum Ausgleich der Beinlangendifferenz wurde in der Orthopadischen Universitatsklinik Nijmegen ein Schuh mit verdickter Sohle angefertigt Von der Abteilung für Plastische Chirurgie wurde der Rat gegeben, die besonders hinderlichen Hautveranderungen an der rechten Fußsohle operativ entfernen zu lassen, wobei der Defekt mit Spalthaut gedeckt werden sollte Zunachst machten wir jedoch einen konservativen Behandlungsversuch mit

Acitretin 20 mg/Tag (0,5 mg/kg KG). Innerhalb von 4 Monaten bildeten sich die Hautveränderungen an den Fußsohlen so weit zurück, daß das Mädchen wesentlich leichter gehen konnte. An den übrigen Körperstellen blieb der ichthyosiforme Nävus jedoch unbeeinflußt und breitete sich eher noch aus.

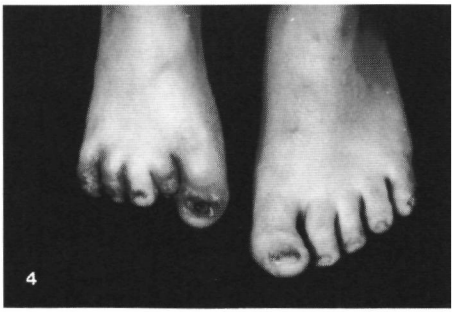




**Abb. 1.** CHILD-syndrom. Ichthyosiformer Nävus und fehlende Schambehaarung mit scharfer Begrenzung in der Medianlinie.

**Abb. 2.** CHILD-syndrom. Ichthyosiformer Nävus mit gelber wachsartiger Schuppung in der rechten Kniekehle.

**Abb. 3.** CHILD-syndrom. Dysplasie der rechten Hand mit Unregelmäßig verkürzten Fingern und Onychodystrophie.

**Abb. 4.** CHILD-syndrom. Dysplasie des rechten Fußes mit Hypotrichose, Onychodystrophie und nässenden, papillomatösen Proliferationen in den Interdigitalräumen.

*Patientin 2 (Mutter)*

Die 44jährige Frau ist von guter Gesundheit. Es ist keine Asymmetrie des Körperbaus erkennbar. Ab dem 5. Lebensjahr haben sich streifenförmige ichthyosiforme Hautveränderungen am Kinn sowie am Mittel- und am Ringfinger der linken Hand entwickelt. Diese hyperkeratotischen Areale sind von einem von uns (D.K) über mehrere

Jahre hinweg beobachtet worden Eine dritte lineare ichthyosiforme Hautveranderung ist ab dem 13 Lebensjahr am Mons pubis aufgetreten Sie verlief, ebenso wie die Lasion am Kinn, rechts von der Medianlinie und parallel zu ihr Alle 3 navoiden Hautveranderungen haben sich um das 20 Lebensjahr herum spontan zuruckgebildet

*Befund*

Am Kinn und am Mons pubis bestehen streifenformige haarlose Areale dort, wo fruher die hyperkeratotischen Hautveranderungen vorhanden waren Der linke Mittelfinger weist einen streifenformigen Nageldefekt vom Typ der Onychorrhexis auf, die radiare Halfte der Lunula zeigt eine unregelmaßige Kontur

*Besprechung*

Bei der Tochter ist die klinische Symptomatik so typisch, daß kein Zweifel an der Diagnose bestehen kann Die Beobachtung macht deutlich, warum es besser ist, die charakteristischen Hautveranderungen als ichthyosiformen Navus zu beschreiben und nicht als ichthyosiforme Erythrodermie wie ursprunglich von uns vorgeschlagen [6 8] Eine Agenesie der ipsilateralen Niere war bisher zweimal beobachtet worden [7 15] Ein bislang noch wenig beachteter Befund ist das Fehlen der Korperhaare in streifenformiger Verteilung entsprechend dem System der Blaschko-Linien

Mit dieser Beobachtung haben wir zum ersten Mal die Vererbung der charakteristischen Hautveranderungen von der Mutter auf die Tochter nachgewiesen, wie es aufgrund des atiologischen Konzeptes zu erwarten ist Bedeutsam erscheint uns die Tatsache, daß bei der Mutter nur geringgradige Hautveranderungen bestanden Erst nachdem wir die Mutter, die selbst Naturwissenschaftlerin ist, uber den X-chromosomal-dominanten Erbgang mit Letalwirkung für mannliche Embryonen und uber das Phanomen der X-Inaktivierung mit der Moglichkeit einer extremen Lyonisierung [6] unterrichtet hatten, erzahlte sie uns, daß bei ihr in der Jugendzeit streifenformige hyperkeratotische Hautveranderungen an 3 verschiedenen Korperstellen bestanden hatten, und machte uns auf die Nagelveranderung als Restzustand nacht spontaner Ruckbildung dieser navoiden Lasionen aufmerksam Die Krankheit der Tochter hatte somit sehr leicht irrtumlich als sporadisch angesehen werden konnen

Die Beobachtung ist insofern bedeutsam, als sie alle bisher mitgeteilten sporadischen Falle in einem neuen Licht erscheinen laßt Ohne eine sehr sorgfaltige Untersuchung der Mutter bestehen Zweifel am sporadischen Auftreten des Syndroms Dabei sollte man auch an die Moglichkeit extrakutaner Minimalsymptome denken So haben Schlenzka et al [19] eine leichte Verkurzung des linken Armes und Beines bei der Mutter eines Kindes mit CHILD-Syndrom beschrieben Bei den fruheren unzweifelhaften Familienbeobachtungen [2 10 14] wurden die Mutter als erscheinungsfrei geschildert Ohne eine gezielte Untersuchung der Haut und des Skelettes kann man jedoch nicht mit Sicherheit davon ausgehen, das sie phanotypisch gesund waren

Die Dinge liegen beim CHILD-Syndrom somit ähnlich wie bei der X-chromosomal-dominanten Chondrodysplasia punctata. Nachdem es gelungen war, ausgehend von der Geschlechtsverteilung und dem Mosaikmuster der Hautanomalien den Erbgang aufzudecken[7], haben andere Autoren ihre "sporadischen" Beobachtungen nachuntersucht und bei den Müttern charakteristische Hautanomalien in Mosaikartiger Verteilung gefunden, wie es beim X-chromosomal-dominanten Erbgang zu erwarten ist.[12]

Auch beim CHILD-Syndrom werden solche Familienbeobachtungen zunehmen. Das die Kenntnis dieser Krankheit noch ungenügend ist, kann nicht verwundern, denn das Syndrom wird bisher in den dermatologischen Standardwerken mit keinem Wort erwähnt.[1,3,18] Wir nehmen an, das die Genodermatose bislang unter folgenden Fehldiagnosen archiviert wird, denn unter diesen Bezeichnungen finden wir das CHILD-Syndrom bis auf den heutigen Tag auch in der Literatur beschrieben: ILVEN, Epidermalnävus-Syndrom, Solomon-Syndrom, halbseitige Psoriasis, atypische Erythrokeratodermie, Conradi-Syndrom.[9,11,16,20,4,17]

Das die Mutter meist sehr viel milder befallen sein wird als die Tochter, läßt sich damit erklären, das die Fähigkeit zur Fortpflanzung umso größer ist, je geringer der Phänotyp ausgeprägt ist, und das sich andererseits das CHILD-Syndrom umso leichter erkennen läßt, je schwerer die Patientin betroffen ist.

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# Chapter 6

# Co-occurrence of Linear Psoriasis and Porokeratotic Eccrine Ostial and Dermal Duct Naevus

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PM Steijlen
R Happle

Acta Derm Venereol (Stockh) 1993; 73: 311-312

In a previous communication a case report was presented on inflammatory linear verrucous epidermal naevus (ILVEN).[1] In this letter we report on some additional features which are of relevance for the understanding of ILVEN.

At the age of 12 years the patient, a 47-year-old man, had experienced an itching linear erythematosquamous lesion on his left shin, the left thigh, the dorsal aspect of the left lower leg, the genital region, the lumbar region and also on the trunk. The distribution pattern followed the lines of Blaschko as described in the previous publication.[1] The lesions on the left lower leg had a partly verrucous appearance. In addition to this linear pattern, erythematosquamous plaques with a typical psoriatic appearance were observed at the extensor aspects of both elbows and the scalp; the finger nails showed multiple pits. At careful examination we observed as an extension of the linear arrangement of erythematosquamous lesions at the left lower leg, discrete hyperkeratotic papules and pits at the plantar and lateral aspect of the left foot. More proximally at the lateral surface of the foot these lesions partly adopted a psoriatic appearance (Figure 1a-b). By contrast, the remaining erythematosquamous lesions within the mosaic pattern and the classical psoriatic plaques did not show any hyperkeratotic papules and pits but had the typical appearance of psoriasis. It is not known for how many years these hyperkeratotic papules and pits have been present. There was no family history of similar skin disorders. The response of the erythematosquamous lesions of this patient to an antipsoriatic treatment has been described before.[1] Topical corticosteroids, tar, UVB and PUVA did not improve the lesions, whereas short-contact dithranol treatment resulted in virtually total clearing of the erythematosquamous lesions after 8 weeks. The lesions, however, relapsed within 6 months' treatment. Dithranol has been reinstituted on various occasions for the past 3 years.

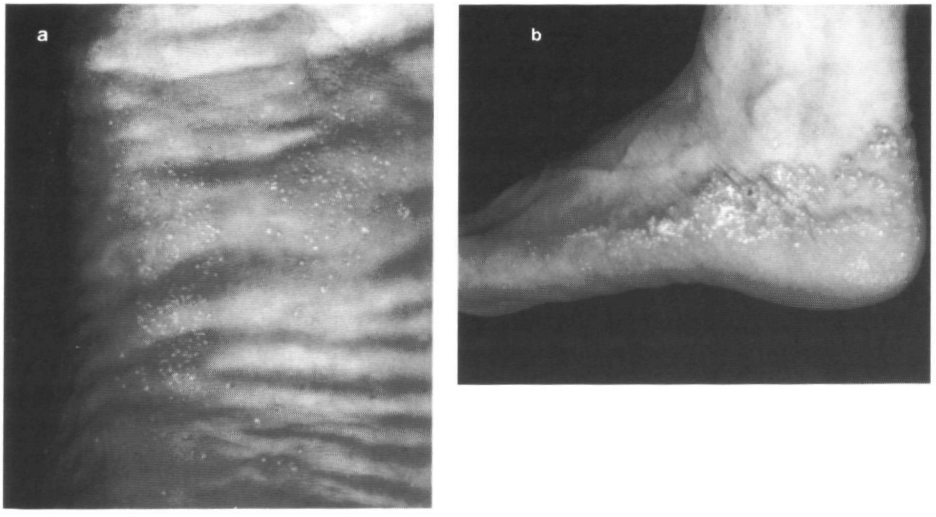


**Fig. 1.** Porokeratotic Eccrine Ostial and Dermal Duct Naevus. **a.** Discrete papules and pits on the left sole. **b.** Hyperkeratotic papules coalescing with a psoriasiform lesion.

Histopathological examination of biopsies taken from the erythematosquamous lesions on the left lower leg and thigh showed features typical of psoriasis: acanthotic epidermis with elongation of the rete ridges, hyperkeratosis of the ortho and para type, dermal accumulation of mononuclear cells and polymorphonuclear leukocytes, and intra-epidermal micro-abscesses filled with polymorphonuclear leukocytes. The eccrine glands did not show any abnormality. The histological appearance of a hyperkeratotic lesion on the left sole was quite different. The epidermis was acanthotic, with a pronounced granular layer and orthokeratosis. Dilated eccrine ducts were sleeved by a parakeratotic cornoid lamella (Figure 2). The glandular portions of the eccrine glands were not involved.

**Fig. 2.** Histopathological appearance of a papule on the left sole: dilated eccrine duct sleeved by a porokeratotic cornoid lamella.

Clinically and histologically the lesions on the left foot represent porokeratotic eccrine ostial and dermal duct (PEODD) naevus. PEODD naevus is a dermatosis that follows the lines of Blaschko. The naevus is characterized by porokeratotic plugs related to eccrine sweat ducts.[2-5] This naevus can be best explained by a somatic mutation. In cases reported so far the lesions had been present since birth.[2-5] In our patient the lesions were rather

discrete and may have gone unnoticed at birth Linear psoriasis superimposing linear verrucous epidermal naevus has been observed by several authors.[6-8] Bondi reported a case of linear psoriasis extending beyond the boundaries of linear verrucous epidermal naevus, associated with bilateral psoriatic lesions of the common type [9] In the present case, at the lateral surface of the foot the topographical coexistence of linear psoriasis and PEODD naevus was evident. PEODD naevus may trigger psoriasis as a "Köbner" phenomenon. In the present case, however, linear psoriasis is extended beyond the areas clinically involved by PEODD naevus. This unusual observation can be best explained in the following way. PEODD naevus is caused by a specific somatic mutation. Within the systematized linear skin area representing a clonal outgrowth of cells carrying this mutation, the phenotype is only partially expressed. In other parts, the linear skin areas look normal but form a *locus minoris resistentiae*, predisposing to the development of psoriasis. In other words, the mutation responsible for PEODD naevus would constitute a major psoriasis gene within a spectrum of polygenic predisposition for psoriasis. Other explanations, such as the assumption of a contiguous gene defect with deletion of a neighbouring psoriasis locus, appear to be less likely. Whatever explanation holds true, such clinical observations may help in the future to elucidate the enigmatic aetiology of psoriasis.

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# Part II

# Cell Biological Aspects

# Introduction

Based on biochemical and cell biological studies attempts have been made to simplify classification of ichthyosis and other monogenic disorders of keratinization. Using histologic and cell kinetic studies, Frost and co-workers classified the ichthyoses into hyperproliferation hyperkeratoses and retention hyperkeratoses.[1]

A drawback of this classification is that many entities are lumped together and that for many disorders of keratinization such an investigation has not yet been done. Besides the barrier function of epidermal lipids it is known that epidermal lipid metabolism plays an important role in the homeostasis of the stratum corneum. Alterations in epidermal lipids were detected in X-linked recessive ichthyosis, Refsum's disease and neutral lipid storage disease. Williams and Elias demonstrated that the clinical heterogeneity of lamellar ichthyosis was reflected in the alkane content of the stratum corneum.[2,3]

Since these alkanes are probably exogenous their conclusions are now under debate. Epidermal lipid research gave only a marginal contribution to the classification of disorders of keratinization until now.

In chapter I data are presented on the keratin expression patterns in a variety of monogenic disorders of keratinization. In particular the question is addressed to what extent these data can be of diagnostic relevance. Since it is known that the dermis also plays a role in epidermal proliferation and differentiation extracellular matrix components were studied in several disorders of keratinization (chapter 2 and 3).

Molecular biology and the expanding knowledge of the human genome made tools available to study disorders of keratinization at the molecular level. Patients with bullous congenital ichthyosiform erythroderma suffer from fragility of the differentiating keratinocytes in the suprabasal layers of the epidermis (epidermolytic hyperkeratosis). This form of ichthyosis is caused by mutations in the genes coding for keratin 1 or keratin 10 that are expressed in the suprabasal keratinocytes.[4-7] Palmoplantar hyperkeratosis of the Vörner type is histologically characterized by epidermolytic hyperkeratosis too This disease is caused by a mutation in the gene coding for keratin 9[8]. Not until recently it was debated whether ichthyosis bullosa of Siemens was an entity distinct from bullous congenital ichthyosiform erythroderma (part 1, chapter 1). In chapter 4 and 5 molecular biological studies are presented which show that ichthyosis bullosa of Siemens is an entity distinct from bullous congenital ichthyosiform erythroderma but that ichthyosis bullosa of Siemens and autosomal dominant ichthyosis exfoliativa are identical.

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# Chapter 1

# Distribution of Keratins in Monogenetic Disorders of Keratinization: an Immunohistochemical Study

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To be submitted

## SUMMARY

Fifty-five punch biopsies from 54 patients suffering from 13 different disorders of keratinization were examined immunohistochemically using a large panel of monoclonal and polyclonal antibodies of known and restricted specificities For comparison we included biopsies from normal skin Disorders of keratinization without substantial inflammation i e autosomal dominant ichthyosis vulgaris, x-linked recessive ichthyosis and non-erythrodermic lamellar ichthyosis yielded more or less the same staining pattern as observed in normal epidermis staining of the basal layer with the antibodies recognizing keratin 5 and keratin 14, suprabasal staining with the antibodies recognizing keratin 1 and 10 Disorders of keratinization associated with substantial inflammation like erythrodermic lamellar ichthyosis, epidermolytic ichthyosis, erythrodermia variabilis, CHILD syndrome and Darier's disease showed an extension of staining with the antibodies recognizing keratin 5 and 14 into the suprabasal zone In these disorders staining with the antibodies recognizing keratin 6, 16, and 17 was observed frequently Although the present study demonstrates that the expression of keratins is distorted in various disorders of keratinization its diagnostic relevance seems limited

## INTRODUCTION

Intermediate filaments of epithelial cells are composed of keratins and normally consist of two or more different keratin polypeptides In human epithelia the keratins are a family of at least 20 distinct polypeptides (numbered 1 to 20 by Moll et al )[1] which can be divided into tow subfamilies, i e the neutral to basic and the acidic keratins, forming heteropolymers [2 3] The composition of the intermediate filaments varies with epithelia lineage and with the stage of differentiation or development This variety is presumably needed to allow different epithelial cells to fulfil different needs, and functions e g cell differentiation, proliferation and migration In the normal human epidermis four major keratins are expressed sequentially during differentiation The basal keratinocytes contain the keratin pairs 5 and 14, while upon differentiation and migration out of the basal cell layer keratin 1 and 10 synthesis occurs

The monogenetic disorders of keratinization form a large and heterogenous group of skin diseases In this group the ichthyoses represent a major part In patients with bullous congenital ichthyosiform erythroderma mutations have been found in the genes coding for keratin 1 or keratin 10 [4 7]

The aim of the present study was to evaluate the keratin expression patterns in a variety of monogenic disorders of keratinization In particular we want to know to what extent these disorders express keratins normally not present in normal adult skin, and to what extent these disorders display different topographical distribution patterns of normally occurring keratins that could be of diagnostic relevance

Punch biopsies obtained from 54 patients suffering from 13 different disorders of keratinization were examined immunohistochemically using a large panel of monoclonal and polyclonal antibodies of known and restricted keratin specificity

## MATERIALS AND METHODS

### Tissue samples

Skin punch biopsies were obtained from patients with disorders of keratinization (Table I) including autosomal dominant ichthyosis vulgaris (ADIV, n=10), X-linked recessive ichthyosis (XRI; n=6), non-erythrodermic autosomal recessive lamellar ichthyosis (NELI; n=13), erythrodermic autosomal recessive lamellar ichthyosis bullosa (ELI; n=5), bullous congenital ichthyosiform erythroderma of Brocq (BCIE; n=5), ichthyosis bullosa of Siemens (IBS; n=4), epidermolytic epidermal naevus (EEN, n=2), harlequin fetus (n=1), CHILD syndrome (n=2), restrictive dermopathy (n=1), Darier's disease (n=2), collodion baby (n=2), and erythrokeratodermia variabilis (EKV; n=1). Biopsies were taken from the most affected regions, snap frozen directly after removal and stored in liquid nitrogen. Additionally, specimens of lesional skin were taken for routine histological examination. Furthermore, in the patients affected with ichthyosis, harlequin fetus, CHILD syndrome, restrictive dermopathy, collodion baby and erythrokeratodermia variabilis, biopsies were taken for electron microscopical examination. Diagnoses were based on the clinical, histological and electron microscopical findings. The diagnosis XRI was based also on the assessment of steroid sulphatase deficiency in leucocytes.[8] For comparison we included frozen biopsies from normal skin (n=9), from psoriasis involved skin (n=6) and uninvolved skin (n=2), dermatitis (n=2) and lichen ruber planus (n=1) (Table I) Patients on systemic and topical treatment with retinoids or glucocorticoids were excluded from this study.

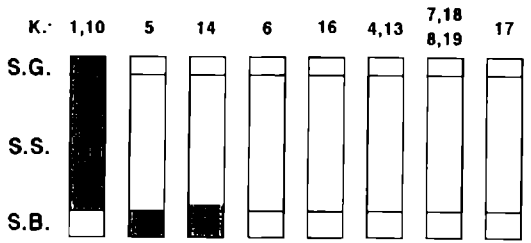
**Table I. Clinical characteristics of patients studied**

| Diagnosis | No of cases | Age (yrs) | Sex | |
|---|---|---|---|---|
| | | | F | M |
| Normal skin | 9 | 30-70 | 4 | 5 |
| ADIV | 10 | ½-67 | 6 | 4 |
| XRI | 6 | 19-58 | 0 | 6 |
| NELI | 13 | 2-66 | 4 | 9 |
| ELI | 5 | ¼-8 | 2 | 3 |
| BCIE | 5 | 1/12-61 | 1 | 4 |
| IBS | 4 | 22-53 | 2 | 2 |
| EEN | 2 | 14, 45 | 1 | 1 |
| Harlequin fetus | 1 | ¼ | 0 | 1 |
| CHILD syndrome | 2 | 12, 16 | 2 | 0 |
| Restrictive dermopathy | 1 | 1/12 | 0 | 1 |
| Darier's disease | 2 | 19, 36 | 2 | 0 |
| Collodion baby | 2 | 1/12 | 2 | 0 |
| Erythrokeratodermia variabilis | 1 | 17 | 1 | 0 |
| Dermatitis | 2 | 50-84 | 1 | 1 |
| Lichen ruber planus | 1 | 59 | 0 | 1 |
| Psoriasis | 6 | 31-60 | 4 | 2 |

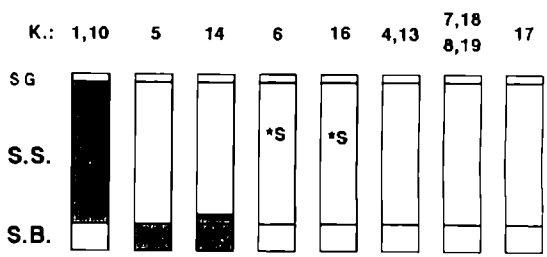
### Antibodies and immunohistochemistry

Specifity, characteristics and references for these antibodies are summarized in Table II

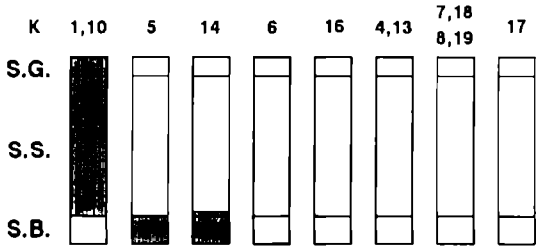
## Normal skin



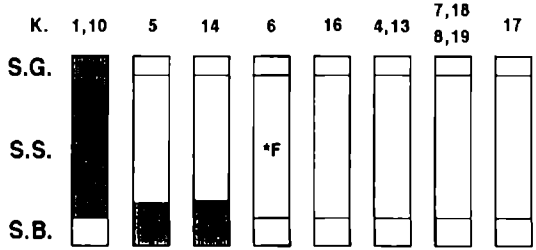

## Autosomal dominant ichthyosis vulgaris




## X-linked recessive ichthyosis




## Non-erythrodermic lamellar ichthyosis



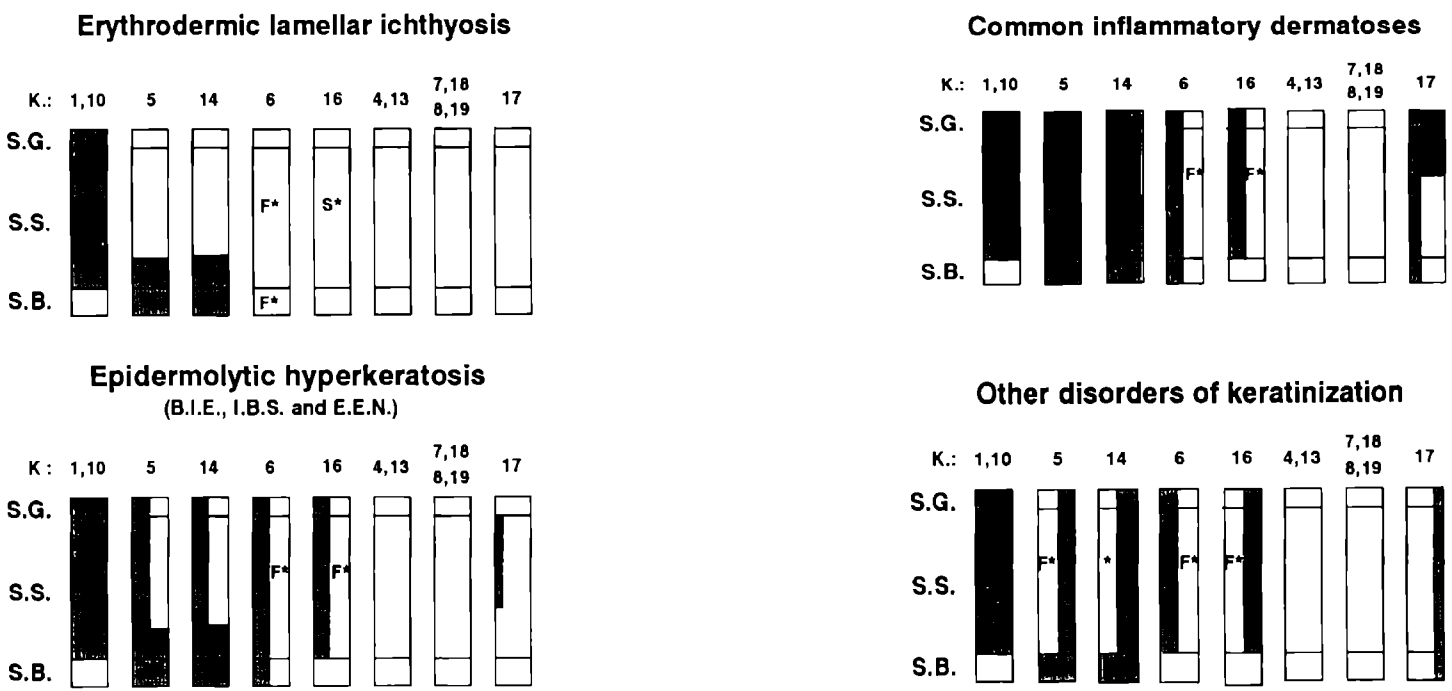



**Fig. 1.** Schematic representation of keratin expression patterns in normal skin, disorders of keratinization and cases of common inflammatory dermatoses For abbreviations see M and M section

SG stratum granulosum SS stratum spinosum SB stratum basale

The individual keratin (K) subtypes indicated by their number, are clustered according to their ability to form pairs

▩ positive staining (>75% cells) □ no staining

F focal staining (10-75% cells) S sporadic staining (1-10% cells) * occasional staining (see results section)

A panel of 19 monoclonal and 2 polyclonal antikeratin antibodies were used in this study. The polyclonal antibodies AF87 and AF124 were a kind of gift from Dr Stuart H. Yuspa (National Cancer Institute, Bethesda, Maryland, USA) These monospecific antibodies were raised in rabbits against C-terminal synthetic peptides of keratins 1 and 6 as described before.[9] The mouse monoclonal antibody KA12, reacting with keratin 6 in two dimensial immunoblots from oesophagus and placenta, was a kind of gift from Dr R. Nagle (Tucson, Arizona, USA).

Four-six micron thick tissue sections were stained with the keratin antibodies using a two-step indirect immunoperoxidase staining procedure as described previously.[19] Exclusively the interfollicular epidermis was assessed for staining.

**Table II.**

| Monoclonal/Polyclonal Antibody | Keratin Specifity | Type of Differentiation | Source and References |
|---|---|---|---|
| AF87 | 1 | suprabasal cells of | D Roop, Bethesda, MD, 9 |
| RKSE60 | 10 | stratifying, cornifying epithelia | Eurodiagnostics, Apeldoorn, NL, 10 |
| RCK102 | 5 (+8) | basal cells | Eurodiagnostics, Apeldoorn, NL, 11 |
| RCK107 | 14 | | F Ramaekers, Maastricht, NL, 12,13 |
| LL002 | 14 | | E Lane, Dundee, UK, 14,15 |
| AF124 | 6 | hyperproliferative | D Roop, Bethesda, MD, 9 |
| KA12 | 6 (+5) | epithelia | R Nagle, Tuscon, AZ, 12 |
| LL025 | 16 | | E Lane, Dundee, UK, 12,16 |
| 6B10 | 4 | suprabasal cells of | Eurodiagnostics, Apeldoorn, NL, 17 |
| 1C7 | 13 | stratifying, non- | Eurodiagnostics, Apeldoorn, NL, 17 |
| 2D7 | 13 | cornifying epithelia | Eurodiagnostics, Apeldoorn, NL, 17 |
| RCK105 | 7 | simple epithelia | Eurodiagnostics, Apeldoorn, NL, 11 |
| LE41 | 8 | | E Lane, Dundee, UK, 18 |
| CAM5 2 | 8 | | Becton&Dickinson, Etten-Leur, NL, 19 |
| M20 | 8 | | G N P van Muijen, Nijmegen, NL, 20 |
| RGE53 | 18 | | Eurodiagnostics, Apeldoorn, NL, 21,22 |
| RCK106 | 18 | | Eurodiagnostics, Apeldoorn, NL, 11 |
| CK18 2 | 18 | | 11 |
| 2C8 | 18 | | G N P van Muijen, Nijmegen, NL,23 |
| E3 | 17 | simple epithelia, some | Dakopatts, Glostrup, DK, 24 |
| RPN1165 | 19 | stratifying epithelia | Amersham, Little Chalfont/Bucks, UK, 25 |

## RESULTS

The distribution pattern of the various keratins as detected in normal skin and in the various lesions are summarized in Figure 1 and depicted in Figure 2

**Normal skin** (Figure 1a)

In normal skin the basal cell compartment contains keratins 5 and 14, while the suprabasal cells express keratins 1 and 10 The keratin 14 antibody RCK107 in general showed staining of the basal, and to some extent the parabasal cell compartment. LL002 was an

exception to this rule, since it stained the whole epidermis. This holds true for all skin diseases described below (staining pattern not shown in the figures)

**X-linked Recessive Ichthyosis (XRI)** (Figure 1b)
The keratin profile in all 6 patients with XRI is similar to that observed in normal skin.

**Autosomal Dominant Ichthyosis Vulgaris (ADIV)** (Figure 1c)
The expression pattern of keratins in ADIV is largely similar to the pattern as seen in normal skin, except for keratin 6 and 16. Keratin 6 expression was observed sporadically in the suprabasal compartment in 4 out of 10 subjects. Only in one of these subjects keratin 16 was expressed sporadically in the suprabasal area. The antibody KA12 and AF124 showed practically identical reaction patterns. This holds true for all diseases described below.

**Non-erythrodermic autosomal recessive Lamellar Ichthyosis (NELI)** (Figure 1d)
A distribution pattern largely similar to that of normal skin was observed. Keratins 5 and 14 were seen in the parabasal cells in 5 out of 13 cases. Keratin 6 was expressed focally in 3 of these cases.

**Erythrodermic autosomal recessive Lamellar Ichthyosis bullosa (ELI)** (Figure 1e)
Remarkably keratin 5 and 14 were seen in several cell-layers above the basal zone. In 4 out of 5 patients basal and suprabasal expression of keratin 6 was observed focally using both antibodies. Moreover, 2 out of 5 patients showed expression of keratin 16.

**Epidermolytic hyperkeratosis (bullous congenital ichthyosiform erythroderma of Brocq (BCIE), ichthyosis bullosa of Siemens (IBS), and epidermolytic epidermal naevus (EEN)** (Figure 1f)
In 3 patients homogeneous staining of keratin 5 and 14 was observed. Five other cases showed an extended expression into the cell-layers just above the basal layer.
In 4 out of 11 patients a homogeneously staining pattern of keratin 6 with varying staining intensity was observed in the entire epidermis. The other patients showed a focal staining for keratin 6. In 3 out of 11 cases keratin 16 expression was homogeneously expressed in the suprabasal compartment. In the other 8 patients a focal staining pattern was observed. Two patients displayed expression of keratin 17 in the suprabasal zone

**Common inflammatory dermatoses** (Figure 1g)
This group of lesions included dermatitis, psoriasis, and lichen planus. Keratin 1 and 10 staining in these cases showed a pattern identical to normal skin Strikingly, immunoreactivity against keratin 5 and 14 was demonstrated in the entire epidermis in all cases Similarly, the antibodies keratin 6 and 16 stained the whole epidermis in 8 cases In

5 cases keratin 17 could be demonstrated in the middle and upper part of the epidermis. In one case the whole epidermis stained with the antibody against keratin 17.

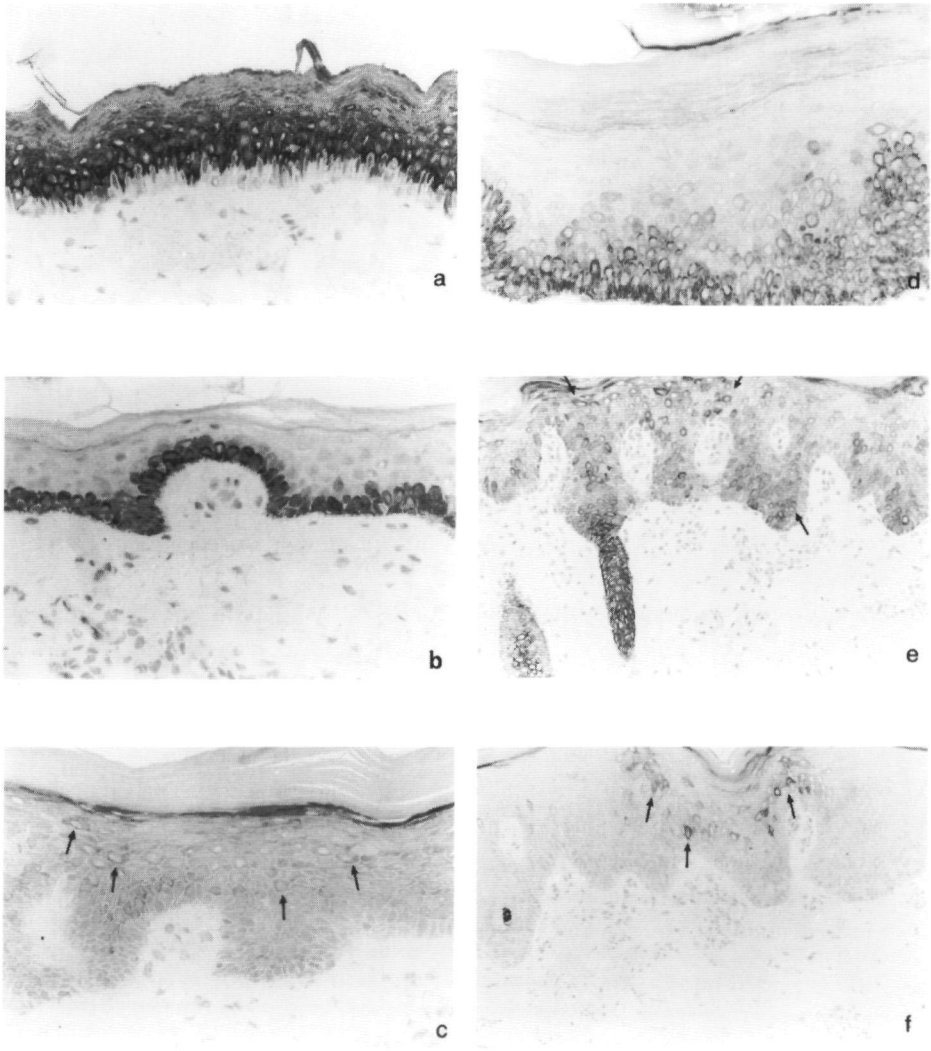



**Fig. 2.** Immuno-perioxidase staining patterns: normal skin incubated with RKSE60 (**a.**); RCK107 (**b.**); non-erythrodermic lamellar ichthyosis incubated with AF124, note focal staining (arrows) (**c.**); erythrodermic lamellar ichthyosis incubated with RCK107 (**d.**); KA12, note focal staining (arrows) (**e.**), and LL0025, note sporadic staining (arrows) (**f.**)

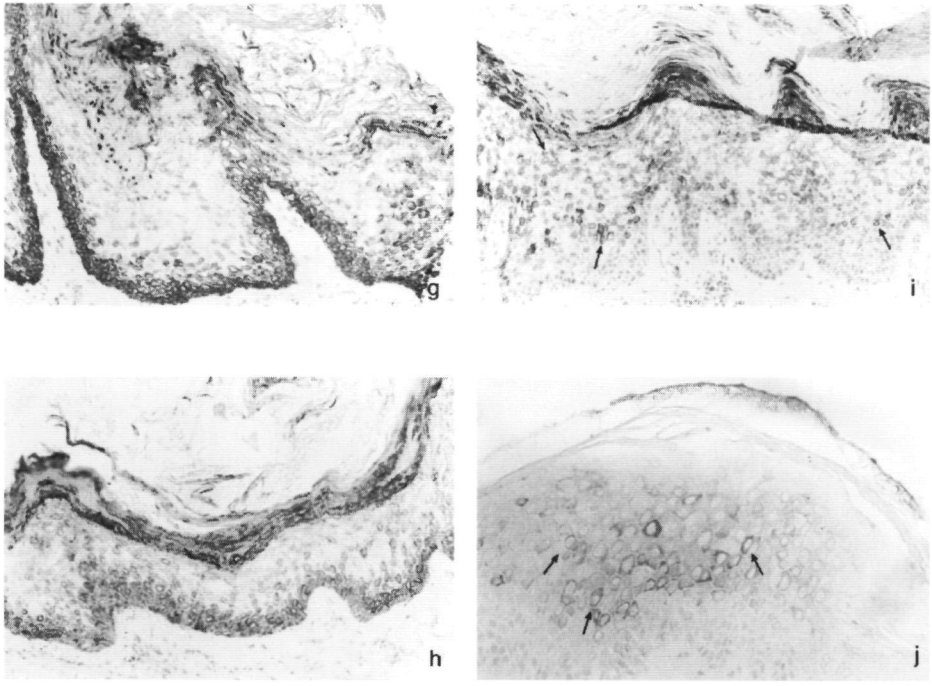



**Fig. 2.** Bullous congenital ichthyosiform erythroderma incubated with RCK107 (**g.**); KA12 (**h.**); LL0025, note focal staining (arrows) (**i.**); ichthyosis bullosa of Siemens incubated with E3, note staining in the upper part of the epidermis (**j.**)

**Other inflammatory disorders of keratinization** (Figure 1h)

This heterogeneous group of lesions included CHILD syndrome, morbus Darier, collodion baby, erythrokeratodermia variabilis, restrictive dermopathy, and harlequin fetus. Staining with antibodies against keratin 1 and 10 showed identical pattern as observed in normal skin in all cases. Staining with antikeratin 5 and 14 was essentially normal, except for collodion baby and erythrokeratodermia variabilis in which the suprabasal cell-layers were also stained. In morbus Darier, collodion baby and erythrokeratodermia variabilis a markedly extensive staining with antikeratin 6 and antikeratin 16 was observed. The remaining disorders in this group showed only focal staining with these antibodies. In one collodion baby keratin 17 expression was noted in the upper part of the epidermis.

The staining pattern of RKSE60 (keratin 10) and AF87 (keratin 1) are superimposable as are the pattern of AF87 and AF124 (keratin 6). The antibody LL002 recognizing keratin 14 stains the whole epidermis in all biopsies. Probably the antibody recognized an epitope

which is preserved during differentiation In some cases the antibody RCK107 shows a more extended expression as compared to the antibody RCK102

## DISCUSSION

The present study using a large panel of antikeratins demonstrates that the expression and topographical distribution of keratins can vary considerably between the various disorders of keratinization The immunohistochemical approach does not permit a quantification of keratin expression, so we will restrict ourselves to the qualitative findings The topographical distribution of keratin 5 an 14 shows an extension of basal expression in normal skin to basal and suprabasal expression in erythrodermic lamellar ichthyosis, epidermolytic hyperkeratosis, collodion baby, erythrokeratodermia variabilis and the group of common inflammation dermatoses Remarkably, these diseases are all characterized clinically and histologically by inflammation The expression of keratin 6, 16 and 17 is pronounced in the same group of diseases whereas these keratins are absent in normal skin Those biopsies which showed expression of keratin 6, showed always a topographical extension of expression of keratin 5 and 14 ADIV, XRI and NELI yielded the same keratin pattern as observed in normal skin However, in those patients suffering from ADIV who demonstrated focal keratin 6 and 16 expression atopic dermatitis may have complicated the situation

Keratin 16 can only be demonstrated in those biopsies which express keratin 6 Keratin 17 is present in those biopsies which express keratin 16 Other disorders of keratinization such as psoriasis, which show clinically and histologically a marked inflammation, demonstrate a marked extension of keratin 5 and keratin 14 expression and an induction of keratin 6, 16 and 17 expression

The regulation of expression of keratin genes appears to be entirely at the level of transcription initiation[26] Modulators at the level of transcription can be various environmental signals, such as hormones, vitamins, growth factors and possibly extracellular matrix components and cell adhesion molecules In how far inflammation factors such as cytokines may be involved is not known The present study is carried out at the protein level, therefore a defect of one of the genes coding for these keratins is not ruled out It is likely that the keratin may be recognized by the antibody although it is functional abnormal Recently, in patients with BCIE (epidermolytic hyperkeratosis) mutations have been found in the genes coding for keratin 1 or keratin 10[47] In our biopsies these keratins will still be recognized by the antibodies of patients with BCIE Therefore, further studies are indicated to discover keratin gene defects within the spectrum of disorders of keratinization

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# Chapter 2

# Tenascin Expression in Human Dermis is Related to Epidermal Proliferation

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## SUMMARY

The extracellular matrix glycoprotein tenascin is sparsely distributed in normal human dermis The authors have shown that in a number of skin diseases (psoriasis, skin tumours), tenascin expression is strongly increased In this immunohistochemical study, using polyclonal and monoclonal antisera, we have tested the hypothesis that tenascin expression in vivo is linked to epidermal proliferation Using the sellotape stripping model in normal human skin, which causes a rapid recruitment of keratinocytes into the cell cycle, induction of tenascin expression was found in the upper dermis within 24 hours after stripping In contrast, in normoproliferative monogenic disorders of keratinization (X-linked recessive ichthyosis, autosomal dominant ichthyosis vulgaris, non-erythrodermic lamellar ichthyosis), no increase in tenascin expression was found compared with normal skin These findings demonstrate a relationship between epidermal proliferation and metabolic alterations in the dermal compartment

## INTRODUCTION

Tenascin is an extracellular matrix glycoprotein[1-4] that is identical to hexabrachion[5], myotendinous antigen[6], cytotactin[7] glioma mesenchymal extracellular matrix antigen[8] and the high molecular weight J1 glycoproteins[8] It is abundantly present in embryonic tissues but the expression is limited in adult tissues In addition, tenascin is reexpressed in connective tissue adjacent to epithelial malignancies[1,10,15] Tenascin is structurally distinct from other extracellular matrix glycoproteins such as fibronectin and laminin and its expression is subject to different control mechanisms[4,15] cDNA clones mapping the cytotactin-tenascin-hexabrachion gene[16-18] showed that there is homology to fibronectin, fibrinogen and epidermal growth factor

Currently, little is known about the function of tenascin It has been shown to promote cartilage differentiation in vitro[3] and it supports the growth of primary tumor cells in serumfree culture medium[9] Tenascin can partially inhibit the attachment of cells to fibronectin[19], but appears to act as a surface adhesion molecule in the central nervous system[9]

The presence of tenascin in normal and injured rat skin[4] and normal and diseased human skin[11,15] has recently been reported In normal human skin, tenascin was detected in the papillary dermis and adjacent to the basement membranes of adnexal structures, using immunohistochemical techniques[11,15] Electron microscopical examination of human skin[11] showed that tenascin is not a basement membrane component but is localized in patches beneath the basal lamina In rat skin, tenascin was detected in a discontinuous distribution near the basement membrane of the dermal-epidermal junction In addition, it was detected near basement membranes of subepidermal capillaries and around hair follicles We have recently studied the expression of tenascin in human skin diseases characterized by epidermal hyperproliferation[15] It was shown that there is a strong increase in tenascin production both in benign and premalignant skin diseases compared with normal skin The aforementioned observations suggest that activated epithelium, during embryonic

differentiation, wound healing, or disease (cancer, psoriasis), induces tenascin expression by the adjacent connective tissue. To obtain support for this hypothesis, we have studied tenascin expression in human dermis using a model for epidermal activation in vivo (the sellotape stripping model). We compared tenascin expression in this model with normal human skin and a number of skin diseases with an intrinsic defect in differentiation rather than in proliferation. In parallel, the expression of keratin 16, a known marker for hyperproliferation in keratinocytes, has been studied.

## MATERIALS AND METHODS

*Antisera*

Antiserum against tenascin purified from rat embryo and chicken fibroblasts cultures was raised in rabbits and purified as described before.[14,15] Both antisera yielded identical staining patterns in all tissues examined. A monoclonal antibody (T2H5) against human tenascin was obtained from a fusion between splenocytes from BALB/c mice, immunized with a human mammary tumor homogenate, and Sp2/0-Ag 14 nonsecreting mouse myeloma cells. The hybridoma supernatants were screened by indirect peroxidase immuno-histochemistry on cryostat sections of human mammary tumors. Evidence that T2H5 detects an epitope of human tenascin was obtained by sequential immunoprecipitation with polyclonal anti-tenascin and Western blotting Immunohistochemical staining with polyclonal anti-tenascin sera gave similar results as for the monoclonal antibody. Monoclonal antibodies against human plasma fibronectin (F7387) and keratin 13/16 (Ks8.12) were obtained from Sigma Chemical Co, Mo, USA. Peroxidase-conjugated antisera to mouse and rabbit immunoglobulins were obtained from Dakopatts, Glostrup, Denmark.

*Biopsies*

Biopsies specimens were taken, after the subjects gave informed consent, either from healthy volunteers or patients from the Dermatology policlinic at the University of Nijmegen. The study included 10 healthy volunteers, 6 patients with autosomal dominant ichthyosis vulgaris (ADIV), 6 patients with X-linked recessive ichthyosis (XRI) and 7 patients with non-erythrodermic lamellar ichthyosis (NELI). Permission from the local committee for experiments on humans was obtained. Punch biopsies (3 mm diameter, 5 mm deep) or shave biopsies (5 mm diameter, 0.3 mm deep) were taken after local anaesthesia. Tissue specimens were snapfrozen in liquid nitrogen and stored at -80°C until further use.

*Stripping procedure*

The sellotape stripping model was used to induce epidermal proliferation in healthy human volunteers as described.[20] Briefly, stratum corneum (from the back) is removed by consecutive applications of sellotape. Removal is complete when the area appears glistening, and the living cell layer is reached. At various time intervals after stripping,

shave or punch biopsies were taken and the tenascin expression was compared with untreated skin, using immunohistochemistry

*Histology*

Cryosections were prepared using standard procedures Immunoperoxidase staining was performed with antisera diluted in Phosphate-buffered saline (PBS), 1 100 for the polyclonal antitenascin sera and 1 500 for the monoclonal antibody, 1 50 for antifibronectin and 1 40 for Ks8 12 (60 minutes at room temperature for all antisera) After being washed in PBS, sections were incubated either with peroxidase-conjugated rabbit anti-mouse Ig or swine anti-rabbit Ig (1 100 dilutions in PBS with 5% normal human serum), 30 minutes at room temperature) The sections were developed using aminoethylcarbazole according to standard procedures

All experiments included appropriate controls such as omission of the primary antibody or the use of non-immune serum

## RESULTS

*Tenascin expression following tape stripping*

Normal healthy volunteers were subjected to tape stripping (day 0) and biopsies were taken at 8 hours and on day 1, 2, 4, 7, 12 and 14 In addition, biopsy specimens were taken from untreated normal skin of all volunteers to determine the basal expression of tenascin In a previous study, we found that tenascin expression in normal skin varies from being almost absent to being present in a patchwise distribution in the papillary dermis [15] Cryosections were stained with monoclonal or polyclonal antisera All antisera (2 polyclonal and 1 monoclonal) gave identical staining patterns Tape stripping of human skin is a model for superficial wounds Only the stratum corneum of the epidermis is removed, the dermis remains fully intact The data on tenascin staining are summarized in Table I Quantification of tenascin staining using the surface area of positive staining in the dermal layer was not attempted since this is strongly affected by the plane of sectioning Therefore we used the criterium whether tenascin staining of the papillary dermis was continuous or patchwise Since in normal skin tenascin expression is nearly always discontinuous (Figures 1a and 2a), the effect of tape stripping is easily noted as a layer of continuous tenascin staining of the papillary dermis along the basal membrane (Figures 1b and 2b, 48 hours after stripping) The expression of fibronectin, a structurally and functionally related glycoprotein which is present diffusely throughout normal dermis, was not found to be significantly increased at 48 hours after stripping (Figure 3a-c) This is in contrast with findings in models for healing of deep dermal wounds, where both tenascin and fibronectin expression are increased The distribution of fibronectin is markedly different from tenascin As already shown by others[21], fibronectin is diffusely distributed in the entire upper dermis, whereas tenascin expression is more sharply demarcated and is usually restricted to the papillary dermis adjacent to the basal membrane In our model, the increased expression of tenascin at 48 hours, as shown in

Figures 1 and 2, was a consistent finding in all 10 healthy volunteers. In addition, a limited number of biopsy specimens were taken at other time points (up to 14 days after tape stripping). The presence of tenascin did not change very much over this period (not shown). Whether this represents continuous expression or slow turnover is not clear.

**Table I. Tenascin expression after tape stripping**

| Time after stripping | Tenascin expression in upper dermis |
|---|---|
| 0 h | Absent or patchy (10/10) |
| 8 h | Absent or patchy (4/4) |
| 24 h | Continuous (5/5) |
| 48 h | Continuous (10/10) |
| 7 days | Continuous (2/2) |
| 14 days | Continuous (4/4) |

Normal healthy volunteers were tape stripped as described. At various intervals punch or shave biopsies were taken and cryosections were stained for tenascin either with monoclonal or polyclonal antibodies, which gave similar results. The expression of tenascin was scored according to the distribution rather than the intensity. An increase in tenascin expression along the entire basal membrane was already seen 24 hours after tape stripping. In parentheses. The number of biopsies performed at each time point and the number of biopsies showing the designated distribution pattern are given in parentheses.

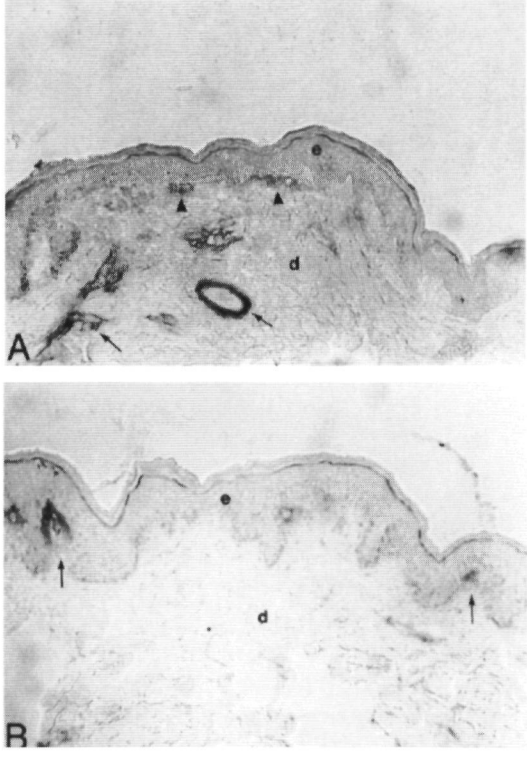


**Fig. 1.**
**a**. Tenascin expression in normal human skin before tape stripping, (punch biopsy, polyclonal anti-serum). Note the sparse staining in the upper dermis (arrow heads). Blood vessels and connective tissue adjacent to adnexal structures are positive (arrows). Magnification x100, d=dermis, e=epidermis. **b.** Tenascin expres-sion in normal human skin before tape stripping, (shave biopsy, monoclonal anti-body). Note the sparse staining in the upper dermis (arrows), magni-fication x100, d=dermis, e=epidermis.

*Tenascin expression in disorders of keratinization*

To investigate the relationship of tenascin expression with keratinocyte proliferation, tenascin expression was studied in diseases with abnormal keratinocyte differentiation which are known to be normoproliferative (ADIV, XRI, NELI). Psoriatic skin, which was previously shown to give strong tenascin staining[15] is shown as a positive control (Figure 4). The results summarized in Table II, indicate that tenascin expression in XRI, ADIV and NELI is comparable to that in normal skin, i.e. staining for tenascin in these diseases was mostly absent or discontinuous. Continuous staining was rare (3 of 19 patients). Figure 5 shows an almost total absence of dermal tenascin staining in XRI. Occasional positive staining was found in the dermal papillae in NELI, as shown in Figure 6.

**Table II. Tenascin expression in disorders of keratinization**

| Disease | Tenascin expression in upper dermis |
|---|---|
| Psoriasis | Continuous (6/6) |
| XLRI | Absent to patchy (6/5) |
| ADIV | Absent to patchy (6/5) |
| NELI | Absent to patchy (7/6) |

Tenascin expression in disorders of keratinization was studied using polyclonal and monoclonal antibodies, which gave identical results. In lesional psoriatic dermis intense staining was found in the entire upper dermis. In the monogenic disorders, tenascin expression was in general similar to that of normal skin. The number of patients studied and the number of patients showing the indicated distribution pattern are given in parentheses.

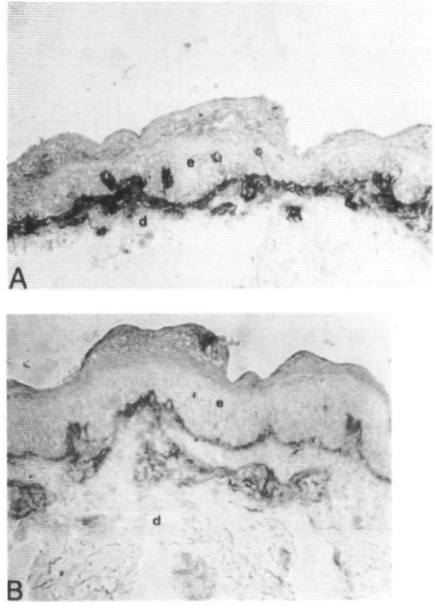


**Fig. 2.**

**a.** Tenascin expression at 48 hours after tape stripping (same individual as in figure 1a, shave biopsy, polyclonal antiserum). Note that the entire upper dermis shows positive staining, magnification x100, d=dermis, e=epidermis.

**b.** Tenascin expression at 48 hours after tape stripping (same individual as in Figure 1b, punch biopsy, monoclonal antibody). Note that the entire upper dermis shows positive staining, magnification x100, d=dermis, e=epidermis.

*Keratin 16 staining*

The suprabasal expression of keratin 16 in human epidermis is a known marker for keratinocyte hyperproliferation. Previous studies have shown that keratin 16 expression is induced in psoriasis and after tape stripping[20], as shown by staining with the monoclonal antibody Ks8.12. In order to confirm the non-hyperproliferative nature of the XRI, ADIV and NELI, we investigated keratin 16 expression in these diseases since no published data are available. Keratin 16 expression was found to be similar to that of normal epidermis i.e. only the basal layer showed positive staining. Suprabasal staining was not seen in any of the XRI (Figure 7) or ADIV patients (not shown). In skin from only one of the NELI patients (not shown) focal suprabasal staining was seen. Thus, in nearly all cases a positive correlation between dermal tenascin staining and suprabasal keratin 16 expression was found.

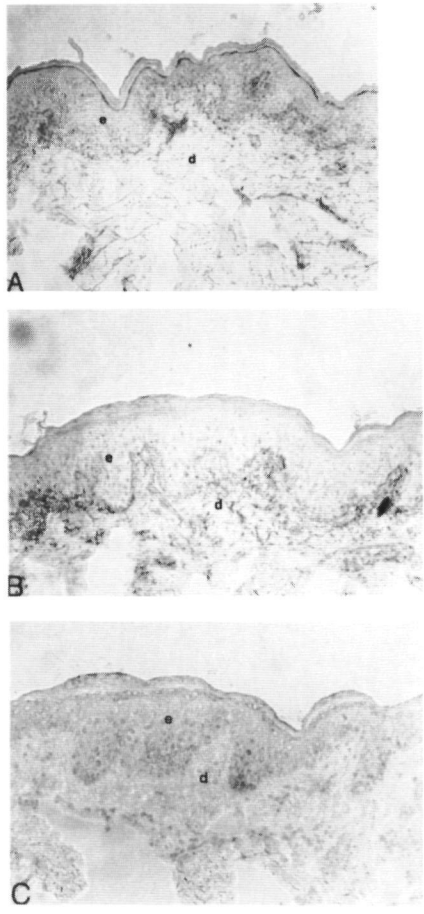


**Fig. 3.**

**a.** Fibronectin expression in normal skin before tape stripping, same individual as in figures 1b and 2b, shave biopsy). Note the diffuse, reticular staining of fibronectin in the upper part of the dermis, magnification x100, d=dermis, e=epidermis.

**b.** Fibronectin expression at 48 hours after tape stripping (same individual as in figure 3a, shave biopsy). The distribution and intensity of staining is similar to that of normal skin, magnification x100, d=dermis, e=epidermis.

**c.** Control section of tape stripped skin at 48 hours (same individual as figure 3a and 3b, shave biopsy), showing complete absence of staining, magnification x100, d=dermis, e=epidermis.

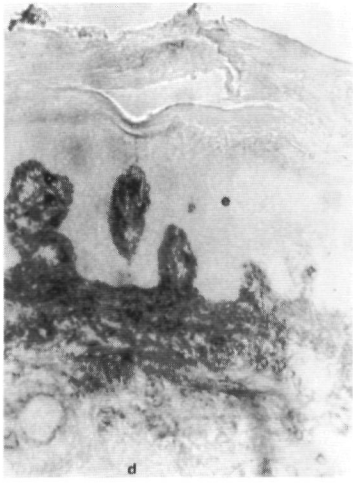


**Fig. 4.** Tenascin staining in lesional psoriatic skin (punch biopsy, monoclonal antibody). Note the intense continuous staining of the entire upper dermis, magnification x 100, d=dermis, e=epidermis.

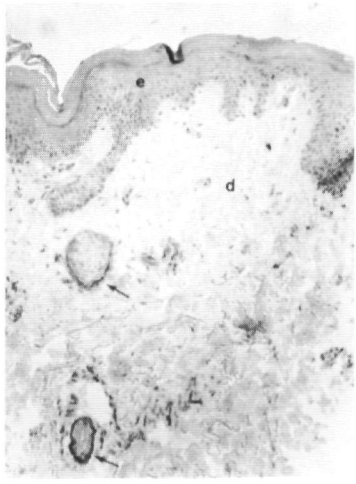


**Fig. 5.** Tenascin staining in ADIV (punch biopsy, monoclonal antibody). Note positive staining around bloodvessels and adnexal structures (arrows). Staining of the upper dermis is weak to absent, similar to normal skin, magnification x 100, d=dermis, e=epidermis.


**Fig. 6.** Tenascin staining in NELI (punch biopsy, monoclonal antibody) showing expression in the dermal papillae (arrows). Note that the expression is discontinuous, similar to normal skin, magnification x 100, d=dermis, e=epidermis.

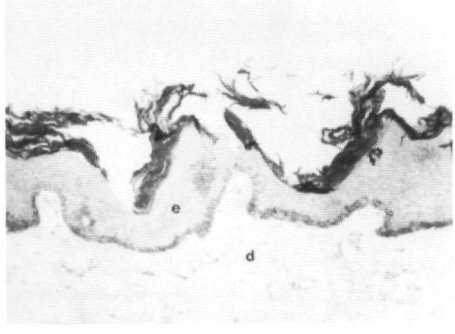


**Fig. 7.** Keratin 16 expression in XRI (punch biopsy). Only the basal cells of the epidermis show positive staining. Staining of the stratum corneum is nonspecific, magnification x 100, d=dermis, e=epidermis.

## DISCUSSION

We have tested the hypothesis that expression of tenascin in human dermis is related to epidermal proliferation In a dynamic in vivo model for the induction of a proliferative response by human keratinocytes, tenascin expression was strongly increased in the dermal compartment In contrast, in a number of diseases characterized by a disturbance of differentiation but with normal epidermal proliferation rates, no increase of tenascin expression was found compared with normal human skin

In normal adult human skin, tenascin is expressed in association with hair follicles, sweat glands, and blood vessels, and in the papillary dermis, although some discrepancies between different studies exist [11 15] Lightner et al [11] were the first to report on the distribution in normal human skin Their findings suggest a higher level of expression in the papillary dermis compared to our previous study[15] and the results of this study This discrepancy is probably caused by differences in location of the skin samples We have noted that the papillary dermis near adnexal structures shows an increased expression of tenascin compared with skin taken from sites with few hairs and sweat glands In our studies, skin was taken from the shoulders which have few adnexal structures, in the study of Lightner et al [11], the skin samples appear to be taken from sites with many adnexal structures

We have recently shown that in hyperproliferative skin disorders such as psoriasis, basal cell carcinoma, Bowen's disease, and solar keratoses, tenascin expression in the upper dermis is greatly increased [15] In the present study, we have used sellotape stripping of human epidermis as a means of inducing keratinocyte hyperproliferation to study the dermal response It has previously been shown that in this model a recruitment of $G_0$-cells into the cell cycle is induced within 24 hours of removal of the stratum corneum [21] In addition, the expression of keratin 16, characteristic of a hyperproliferative phenotype, is induced This study shows that rapid changes in the dermal compartment can also be found As early as 24 hours after tape stripping, an increase in tenascin expression can be demonstrated The expression of fibronectin, a related extracellular matrix glycoprotein, was not increased However, an increase in fibronectin expression is more difficult to see since it is already strongly present in normal skin These observations, together with the observation that in normoproliferative disorders of keratinization (ADIV, XRI, NELI) tenascin expression is not altered, suggest that tenascin is an extracellular matrix component that is specifically increased in response to epidermal activation An additional argument for epidermal involvement in the expression of tenascin by the dermis, is the observation that tenascin expression under non-hyperproliferative conditions (e g , normal skin) is often most pronounced in the dermal papillae (Figure 1b) The function of tenascin expression in the dermis is currently unknown Speculatively, we propose that it functions as a substrate adhesion molecule in the development and regeneration of epidermal and adnexal structures Therefore, tenascin expression could be important in woundhealing processes as suggested by studies in rats [4] The sellotape stripping model is obviously not an ideal model for wound healing since it leaves the basal membrane and

the dermis intact. It is, however, a useful model to study the dermal response to epidermal injury A striking difference with healing of deeper wounds is that in the tape stripping model, no significant increase of dermal fibronectin expression is seen. This phenomenon indicates that tenascin and fibronectin expression are subjected to distinct control mechanisms

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# Chapter 3

# Expression of Tenascin, Biglycan and Decorin in Disorders of Keratinization

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## SUMMARY

The distribution of three (recently discovered) extracellular matrix components (tenascin biglycan and decorin) was studied in normal adult human skin and in a number of monogenic disorders of keratinization, using immunohistology The expression of tenascin which is sparsely distributed in normal human dermis, was found to be grossly increased in epidermolytic hyperkeratoses and in Darier's disease Tenascin expression in three types of ichthyosis (X-linked recessive ichthyosis, autosomal dominant ichthyosis vulgaris, non erythrodermic lamellar ichthyosis) was similar to that of normal skin The presence of biglycan and decorin did not show a marked variation between the different disorders studied, suggesting that their expression is subject to regulatory mechanisms distinct from those of tenascin

The increased expression of tenascin in two disorders of keratinization with a hyperproliferative phenotype, lends further support to the hypothesis that dermal tenascin expression is increased as a result of epidermal hyperproliferation

## INTRODUCTION

Human skin contains a number of extracellular matrix components which contribute to the mechanical properties of the tissue (e g elastin, the major collagens, large aggregating proteoglycans), or exert regulatory functions with regard to cellular processes such as adhesion, migration, growth and differentiation (e g fibronectin and laminin) Recently, several new extracelullar matrix components have been identified in human skin It was shown that the glycoprotein tenascin (also known under various other names including cytotactin[1], hexabrachion[2] and myotendinous antigen[3]), which is predominantly found in embryonic tissues and tumor stroma, is also expressed in normal human skin [4-6] It was found in low levels in the papillary dermis, and abundantly near hair follicles and sweat gland ducts The expression level in the papillary dermis was found to be strongly increased in hyperproliferative conditions such as psoriasis and epidermal tumors [4-5-7]

The small non-aggregating proteoglycans biglycan (also known as PG-I) and decorin (also known as PG-II), have been found in various connective tissues[8-9], and have recently been demonstrated in fetal and adult human skin [10-11] The expression patterns of tenascin, biglycan and decorin appear to be developmentally regulated in the tissues studied, suggesting that these molecules might be of interest in dermo-epidermal interactions leading to pattern formation and regulation of growth and differentiation Because the monogenic disorders of keratinization represent a heterogenous group of skin diseases with disturbances at the level of epidermal differentiation, we were interested to see whether there is a differential effect on the expression of extracellular matrix components

## METHODS

*Tissues*

Biopsies were taken after informed consent, either from healthy volunteers or patients from the department of dermatology at the University Hospital of Nijmegen The study

included healthy volunteers, and patients with autosomal dominant ichthyosis vulgaris (ADIV), X-linked recessive ichthyosis (XRI), non-erythrodermic lamellar ichthyosis (NELI), epidermolytic hyperkeratoses (including the Brocq type, the Siemens type, and the naevoid type), and Darier's disease Punch biopsies (4 mm diameter, 5 mm deep) or shave biopsies (5 mm diameter, 0 3 mm deep) were taken under local anaesthesic Tissue specimens for cryosectioning were snap frozen in liquid nitrogen and stored at -80°C until further use Tissue specimens for routine histology and for immunohistology on paraffin sections were fixed in 0 4% buffered formalin

*Antisera*

A monoclonal antibody (T2H5) against human tenascin was prepared as described previously[7]. An antiserum against a synthetic peptide corresponding to residues 11-24 of the secreted form of biglycan was raised in a rabbit, after conjugating the peptide with bovine serum albumin [12] Repeated booster injections resulted in an antiserum which exhibited a similar titre as the decorin antiserum in an enzyme-linked immunosorbent assay The decorin antiserum was raised against a fully deglycosylated core protein purified from human fibroblast secretions [13] The applicability of the latter two antisera for staining the proteoglycans in human skeletal tissues has previously been ascertained [14] As controls for the polyclonal sera we used preimmune sera from the same rabbits

Peroxidase-conjugated antisera to mouse and rabbit immunoglobulins were obtained from Dakopatts, Glostrup, Denmark

*Immunohistology*

Cryosections and paraffin sections were prepared using standard procedures Sections used for staining of decorin and biglycan were pretreated with chondroitin ABC-lyase (ICN Biochemicals, Covina, CA, USA) at 0 625 u/ml for 20 min at 37°C, in a 0 1 M Tris buffer with 0 05 M sodium acetate and 0 1% BSA, pH 8 0 This method was adapted from Bianco et al [10]

Immunoperoxidase staining, according to standard protocols, was performed with the T2H5 antibody diluted 1 500, 1 100 for the antidecorin serum, and 1 50 for the antibiglycan serum Sections were incubated either with peroxidase-conjugated rabbit anti-mouse Ig or porcine anti-rabbit Ig (1 100 dilutions in PBS with 5% normal human AB serum), for 30 min at room temperature The sections were developed using aminoethylcarbazole, according to standard procedures

All experiments included appropriate controls, such as omission of the primary antibody or the use of pre-immune serum

## RESULTS

*Tenascin*

Table I summarizes the data found for tenascin In the epidermolytic ichthyoses, the Siemens and Brocq type, and the naevoid type are grouped together The other diseases

comprise ADIV, XRI, NELI and Darier's disease. As described in previous studies, normal skin usually shows a patchy to negative staining for tenascin (Figure 1). A continuous, and generally strong staining was seen in the papillary dermis of the epidermolytic disorders (Figures 2 and 3) and in Darier's disease (Figure 4). In XRI, tenascin staining was mostly absent, whereas in NELI and ADIV, tenascin staining was stronger than in XRI but in general the pattern and intensity mostly resembled that of normal skin.

**Table I. Distribution and intensity of tenascin staining**

| Disease | $N_t$ | Distribution | N | Intensity (mean ± SD) |
|---|---|---|---|---|
| ADIV | 7 | negative | 3 | 0 9 ± 0 9 |
| | | patchy | 2 | |
| | | continuous | 2 | |
| XRI | 5 | negative | 4 | 0 2 ± 0 4 |
| | | patchy | 1 | |
| | | continuous | 0 | |
| NELI | 9 | negative | 1 | 1 0 ± 0 5 |
| | | patchy | 6 | |
| | | continuous | 2 | |
| Epidermolytic ichthyoses | 8 | negative | 0 | 2 0 ± 0 5* |
| | | patchy | 2 | |
| | | continuous | 6 | |
| Darier's disease | 3 | negative | 0 | 2 3 ± 0.6# |
| | | patchy | 1 | |
| | | continuous | 2 | |
| Normal skin | 7 | negative | 2 | 0 8 ± 0 5 |
| | | patchy | 5 | |
| | | continuous | 0 | |

Distribution and intensity of tenascin staining was scored semi-quantitatively on frozen sections as indicated in Methods
$N_t$= total number of patients studied N= number of patients with the distribution pattern * Significantly different from normal skin ($P<0.001$), # significantly different from normal skin ($P<0.05$), Wilcoxon rank-sum test

*Biglycan*

Biglycan core protein was localized in the upper part of the epidermis (stratum spinosum and granulosum). The staining pattern, however, was extremely variable. It ranged from diffuse cytoplasmic staining to more sharply localized staining beneath the plasma membrane, sometimes the entire differentiated compartment of the epidermis was positive, in other cases only a focal expression was found (Figures 5 and 6). Whatever the pattern or intensity of staining was in the suprabasal layer, the basal layer was always negative The papillary dermis showed a continuous staining along the basal membrane (Figures 5 and 6). The reticular dermis was virtually negative except for a variable staining of blood vessels and sweat glands The connective tissue sheath around hair follicles was found to

be positive. The indicated staining patterns were found both in normal adult and fetal skin, and in several types of keratinization disorders studied (ADIV, XRI, NELI, epidermolytic hyperkeratoses). Staining of the hair follicle connective tissue sheath appeared to be more intense in fetal skin than in adult skin.

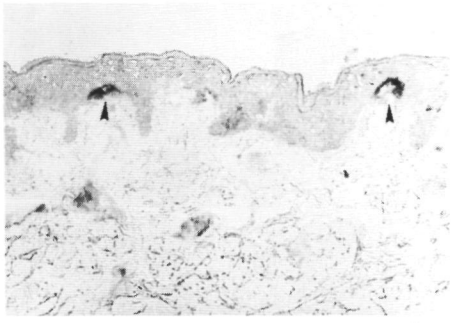

**Fig. 1.** Tenascin expression in normal skin. Note the sparse staining in the upper papillary dermis.

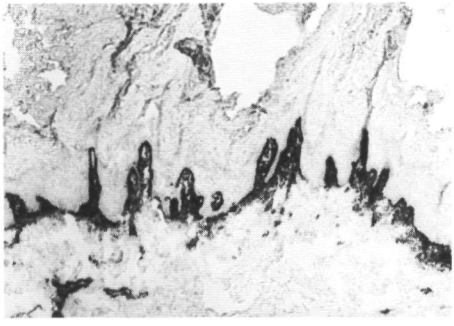

**Fig. 2.** Tenascin expression in epidermolytic ichthyosis of the Brocq type. Note that the entire upper dermis shows positive staining.

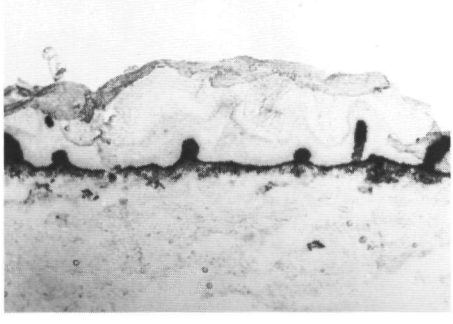

**Fig. 3.** Tenascin expression in epidermolytic ichthyosis of the Siemens type. Note that the entire upper dermis shows positive staining.

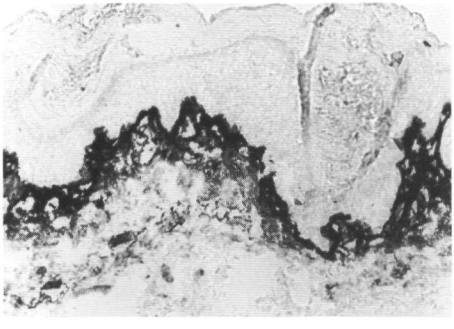

**Fig. 4.** Tenascin expression in Darier's disease. Note that the entire upper dermis shows positive staining.

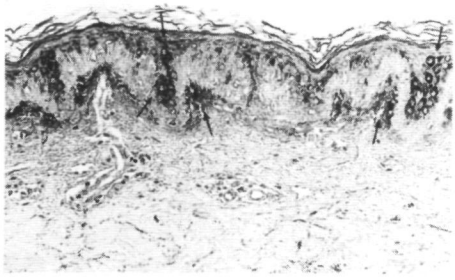

**Fig. 5.** Biglycan expression in normal skin. Note that the suprabasal compartment shows focal staining. The papillary dermis shows positive staining.

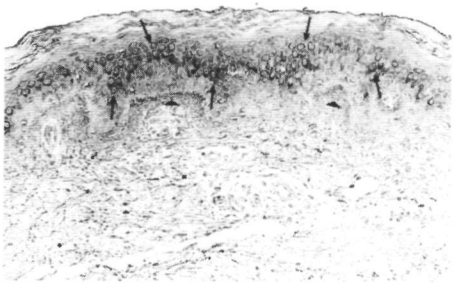

**Fig. 6.** Biglycan expression in autosomal dominant ichthyosis. Note that the suprabasal compartment of the epidermis shows cytoplasmatic staining. The papillary dermis stains mildly.

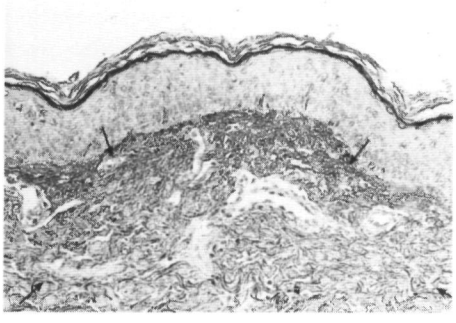

**Fig. 7.** Decorin expression in normal skin. Note that the entire dermis shows positive staining.

*Decorin*

Decorin core protein was absent in all layers of the epidermis. It was found to be present throughout the dermal tissue, the papillary dermis showing the strongest expression (Figure 7). Staining patterns in normal skin and all diseases studied were similar.

## DISCUSSION

At the dermo-epidermal interface, reciprocal interactions between mesenchymal and epidermal cells take place which can be mediated by soluble factors but also by the binding of cells to extracellular matrix components secreted by either cell type These processes are particularly important in embryonic development, and in adults during wound healing and tumour metastasis Under these conditions the epithelial tissues are actively involved in shaping, restoring or distorting a tissue The differentiation programme of the epidermis during fetal development, wound healing or tumour metastasis is distinct from the normal programme, which among others, involves expression of a defined set of cytokeratins in a homeostatic situation Also the composition of the mesenchymal extracellular matrix during development, wound healing and tumour growth differs from that of the normal adult tissue Spatially restricted expression patterns for many extracellular matrix components (including fibronectin, tenascin, decorin and biglycan) have been described

In this study, we have investigated the expression of three recently discovered extracellular matrix components in a number of monogenic disorders of keratinization The results show that the expression of tenascin by the dermal fibroblasts differs markedly between the diseases studied In epidermolytic hyperkeratoses and in Darier's disease, tenascin expression is grossly increased compared with normal skin In ADIV, XRI and NELI tenascin expression is similar to normal skin, as shown previously [15] An important difference between ADIV, XRI and NELI, and epidermolytic ichthyoses and Darier's disease, is the amount of epidermal destruction In the epidermolytic ichthyoses and Darier's disease, acantholysis and inflammation are prominent features of the pathological process The epidermal keratinocytes display a hyperproliferative phenotype with respect to keratin 16 expression, which is found to be elevated in the suprabasal compartment (P M Steijlen et al unpublished data) In ADIV, XRI and NELI none of these features are present This group of diseases displays a normoproliferative phenotype Thus, increased dermal tenascin expression appears to be a phenomenon primarily associated with epidermal hyperproliferation, as we have shown previously in psoriasis and epidermal tumours [5]

Because of the interpatient variation within a given disease, and the small number of patients in each group, the data do not allow definitive statements on the expression levels of decorin and biglycan in these diseases However, no gross variation in the expression patterns was evident in the material studied The distribution of both decorin and biglycan in frozen sections of normal human skin has been described peviously [10 11] Some descrepancies with our data were noticed, and some additional information was obtained The biglycan antiserum we used, only reacts weakly reacts with blood vessel endothelium in skin, whereas in the study by Fleischmajer et al [11] strong staining was found Both antisera were raised against synthetic peptides, albeit from different epitopes, which may explain the difference In contrast, the reactivity of our biglycan antiserum with the papillary dermis of human skin appears to be stronger than that of Fleischmajer et al [11]

The presence of biglycan in the connective tissue sheath of hair follicles, where it co-localizes with tenascin[6], is a new finding

In conclusion, an increased expression of tenascin was observed in epidermolytic hyperkeratoses and Darier's disease Biglycan and decorin expression do not follow this pattern, indicating that these extracellular matrix components are differentially regulated Although the expression levels of extracellular matrix components are probably not causally linked to the disease process in these dermatoses, the data may provide an insight into dermo-epidermal interactions, and may contribute to the classification of these disorders

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# Chapter 4

# Genetic Linkage of the Keratin Type II Gene Cluster with Ichthyosis Bullosa of Siemens and with Autosomal Dominant Ichthyosis Exfoliativa

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## ABSTRACT

Ichthyosis bullosa of Siemens is an autosomal dominant disease which is characterized by mild hyperkeratosis and blistering Autosomal dominant ichthyosis exfoliativa is a recently described disease with clinical features similar to ichthyosis bullosa of Siemens In contrast to ichthyosis bullosa of Siemens, no histological signs typical for epidermolytic hyperkeratosis are observed Linkage analysis was used to test whether keratin gene mutations might underlie both diseases This analysis showed linkage of both disorders with the region of chromosome 12 in which the keratin type II gene cluster is located The keratin type I gene cluster on chromosome 17 is excluded These data, combined with clinical observations, strongly suggest that the genes coding for keratin 1 or keratin 2e, both expressed in the suprabasal compartment of the epidermis and located in the type II gene cluster, are candidate genes for ichthyosis bullosa of Siemens and ichthyosis exfoliativa

## INTRODUCTION

Epidermolytic hyperkeratosis is a histopathological characteristic of a variety of monogenic keratinization disorders comprising bullous congenital ichthyosiform erythroderma of Brocq (BCIE), epidermolytic palmoplantar keratoderma of Vorner (EPPK), ichthyosis hystrix of Curth-Macklin (IHCM) and ichthyosis bullosa of Siemens (IBS) The term epidermolytic hyperkeratosis is also used to refer to the diseases as such

For two of these disorders there is convincing evidence that they are caused by mutations in keratin genes In patients with BCIE, mutations have been found in the genes coding for keratin 1 (K1) or keratin 10 (K10)[1-4] and in case of EPPK in the gene coding for keratin 9 (K9)[5,6] On the other hand, IHCM seems not to be due to a defect in a keratin, since the loci of both the type I and type II keratin gene cluster (17q12-q21 and 12q11-13, respectively) have been genetically excluded[7] So far, there are no clues about the defect causing IBS

IBS has been described as a bullous form of ichthyosis distinct from BCIE[8-10] In the former disease blistering and hyperkeratosis are milder than in BCIE IBS can be further differentiated from BCIE by the absence of congenital erythroderma Light- and electron microscopical examination show the features of epidermolytic hyperkeratosis consisting of coarse keratohyaline granules, intracellular vacuolization and perinuclear clumping of tonofilaments In IBS these features are confined to the stratum granulosum and the upper part of the stratum spinosum whereas in BCIE these findings are present in the whole suprabasal compartment It has been hypothesized that BCIE and IBS are allelic disorders[11] Autosomal dominant ichthyosis exfoliativa (IE) is a recently described type of bullous ichthyosis Only one family has been published sofar The clinical symptoms of IE are very similar to IBS However the histological features of epidermolytic hyperkeratosis were absent[12] Both IBS and IE show autosomal dominant inheritance

We addressed the question whether IBS and IE are due to keratin gene defects Linkage analysis was performed in the kindred with IBS that has been described previously[8] and in

the kindred with IE, described by Vakilzadeh and Kolde[12] employing polymorphic markers from (the close vicinity of) the type I and II keratin cluster In both families, cosegregation of the disease with the type II keratin gene cluster was observed

## MATERIALS AND METHODS

*Patients*

Family 1 has been reported as suffering from ichthyosis bullosa of Siemens [8] The affected individuals had brownish rimpled hyperkeratosis and superficial blistering since early childhood Blistering was more pronounced during hot and humid weather and could be provoked by mild trauma Erythroderma had never been present in any of the affected individuals Skin lesions were localized especially on the extensor surfaces of arms and legs and around the umbilicus, knees and ankles In the hyperkeratotic regions superficially denuded areas were observable Occasionally, fresh blisters ranging in size from 0 5 to 2 cm appeared Light microscopic examination of multiple biopsies of hyperkeratotic areas showed an acanthotic epidermis with orthohyperkeratosis In the stratum granulosum and the upper part of the stratum spinosum coarse keratohyaline granules, intracellular vacuolization and hyperchromatic pycnotic nuclei were observed (Figure 1a) On ultrastructural examination keratinocytes in the upper spinous layer displayed aggregates of tonofilaments forming V shapes or shells around the nuclei

Family 2 has been reported as suffering from ichthyosis exfoliativa [12] The patients showed dark grey hyperkeratotic lesions with denuded areas Superficial blistering occurred spontaneously especially during the summer but occurred also after trivial trauma There was no history of erythroderma in any of the patients Histological examination of multiple biopsies of hyperkeratotic areas showed an acanthosis and orthohyperkeratosis of the epidermis (Figure 1b) The keratinocytes in the granular and upper spinous layer showed marked oedema with indistinct boundaris The nuclei of the cells were small and hyperchromatic and the cells contained a few small keratohyaline granules The electronmicroscopical findings are described before "the cells of the granular and spinous layer were characterized by marked intracellular oedema, the number of tonofilaments and keratohyaline granules were markedly reduced with no grouping of the tonofilaments"

*Linkage analysis*

DNA of patients was isolated from peripheral blood according to the method of Miller et al [13] For the type II keratin cluster we tested polymorphisms in the genes coding for K1 (KRT1)[14], K4 (KRT4)[15], D12S96 and collagen type IIA1 (Col2A1) [16] A map of the relevant region is given in Figure 2 [GDB, 17] For K1 the polymorphic glycine-rich carboxyl-terminal domain was amplified with the forward (5'-TTTGTTGGGCTGG-AAACGGA-GTT-3') and reverse (5'-ACCCCCAGAGCTGGATCCCC-3') primers 200 ng of genomic DNA were amplified in a 15 µL PCR mix with 10 mM Tris-HCl pH 8 3, 50 mM KCl, 1 5 mM $MgCl_2$, 0 005 mM DTT, 0 001% (w/v) gelatin, 1 5 µCi $\alpha^{32}$PdCTP, 250 µM of each dATP, dGTP, dTTP, 3 0 µMdCTP, 75 ng of each of the

primers, 10% (v/v) glycerol and 3% (v/v) formamide. After an initial denaturation of 5 min at 94 °C, 35 cycles of 1 min 94 °C, 2 min 67 °C and 1.5 min 72 °C were performed. The K4 polymorphism was analysed as described by Wanner et al.[15] and the VNTR in the Col2A1 gene as described by Wu et al.[16] and the marker D12S96 (GDB) according to.[18] To test linkage with the type I keratin gene cluster, a polymorphism in the gene coding for K10 (KRT10)[19] and the nearby loci D17S250[20] and D17S579[21] were analysed. The order of the markers of chromosoom 17q is cen-D17S250-KRT10-D17S579-tel (Figure 2). The genetic distance between D17S250 and D17S579 is about 6 cM.[22] For amplification of the K10 polymorphism the following primers were used: forward (5'-GTTTCGGCGGC-GGCTACG-3') and reverse (5'-AGGAGGACTTGTGGCCTCCGC-3'). The same protocol as for K1 was followed with the exception of the use of radioactively labelled forward primer instead of incorporation of $\alpha^{32}$PdCTP. The amount of dCTP in the reaction mixture was adjusted to 250 µM. The loci D17S250 and D17S579 were analysed as described by Kremer et al.[18]

Statistical analysis of the linkage data was performed with the program Linkage, version 5.1.[23] Penetrance was assumed to be 100%. The disease frequencies of 0.00001 for IBS and 0.00001 for IE were assumed. Allele frequencies given in the John Hopkins Genome Data Base, were used for the calculations. For multipoint analysis the distances between D17S250 and KRT10 and between KRT10 and D17S579 were assumed to be 3cM.[22]

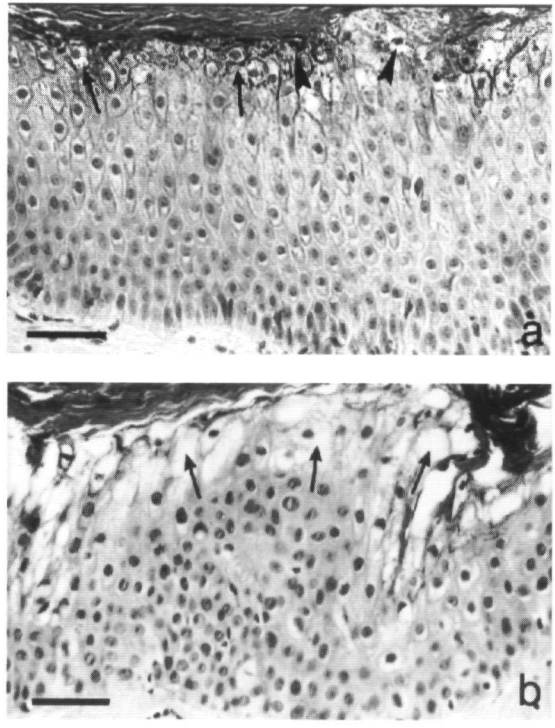


**Fig. 1. a.** Skin biopsy of a hyperkeratotic lesion in a patient from family 1. The epidermis is acanthotic and orthohyperkeratotic. In the stratum granulosum and the upper part of the vacuolization (arrows) spinosum perinuclear and coarse keratohyaline granules are observed (arrow heads).

**b.** Skin biopsy of a hyperkeratotic lesion in a patient from family 2. The epidermis is acanthotic and orthohyperkeratotic. The keratinocytes of the granular and upper spinous layer show marked intracellular oedema and indistinct cellular boundaries (arrows). Bar is 100 µm.

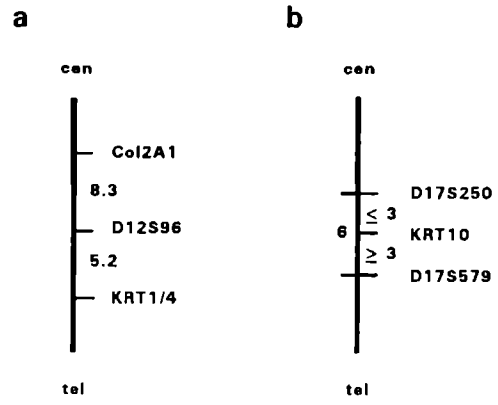


Fig. 2. Map of the loci surrounding the keratin gene cluster on chromosome 12q (a) and on chromosome 17q (b) [GDB, 17,22] Distances are given in cM

## RESULTS AND DISCUSSION

To sort out whether IBS and IE are due to mutations in keratin genes, with K1 and K10 as particularly promising candidates, markers located within, or close to, the keratin gene clusters on chromosome 12q (type II keratins) and 17q (type I keratins) are tested (Figure 2). Haplotypes are depicted in the Figures 3 and 4 and in Table I the pairwise lod scores are given between the markers and IBS and IE.

**Table I. Pairwise lod scores between IBS, IE and loci within or close to the keratin gene clusters. The first three columns give the lod scores calculated for the family with IBS, the last three columns those for the family with IE. Statistical significant lod scores are obtained in both families with the marker Col2A1. The region surrounding the keratin type I cluster on 17q is excluded.**

| | θ | | | | | |
|---|---|---|---|---|---|---|
| | 0 0 | 0 1 | 0 2 | 0 0 | 0 1 | 0 2 |
| Col2A1 | 3 24 | 2 56 | 1 84 | 3 60 | 2 99 | 2 28 |
| D12S96 | 0.63 | 0 52 | 0 41 | 1 48 | 1 21 | 0 93 |
| KRT1 | 1 81 | 1 31 | 0.78 | 0 99 | 0 82 | 0 65 |
| KRT4 | 1 52 | 1 23 | 0.98 | 0 01 | 0 00 | 0 00 |
| D17S250 | ∞ | -0.61 | 0 04 | ∞ | -1 19 | -0.19 |
| KRT10 | ∞ | -0 64 | -0 20 | 1.19 | 0 93 | 0.68 |
| D17S579 | ∞ | -1 99 | -0 84 | ∞ | 0 98 | 1.23 |

In the family with IBS the lod score of 3.24 with Col2A1 clearly shows linkage of this locus and IBS. Both KRT1 and KRT4 are not completely informative in this family.

However, construction of haplotypes (Figure 3) give no indication for recombination between IBS and the region of 12q analyzed with the four markers Col2A1, D12S96, KRT1 and KRT4 These results indicate that IBS is due to a defect in a gene in the analyzed region of chromosome 12q including the keratin genes

KRT 10 shows recombinations with the disease, in the persons IV 8 and V 3 (Figure 3) thereby excluding the K10 gene and other keratin genes in the type I cluster as the cause of IBS in the present family Exclusion of the type I cluster is further substantiated by the lod score of -1 99 at $\theta$=0 1 with D17S579, which is at a distance between 3 and 4 cM from the keratin cluster [22] There also is recombination with D17S250 (Z=-1 95 at $\theta$=0 03) which is located less than 3 cM proximal to KRT10 [22] In a second, small family with IBS comparable results as with the presented family have been obtained However, this family is by itself too small to show statistically significant linkage Combination of lod scores for both families with IBS resulted in the maximum lod score of 4 20 at $\theta$=0 0 with the marker Col2A1

In the family with IE, the lod score of 3 60 with Col2A1 at $\theta$=0 0 indicates linkage between IE and the Col2A1 gene At $\theta$=0 2 the lod score is 2 28 suggesting linkage also with the keratin genes which are at a distance of about 15 cM from the Col2A1 gene [GDB, 17] The three markers closer to or in the keratin gene cluster on chromosome 12 have a very limited informativity (Table I, Figure 4) There is no recombination between IE and these markers The highest lod score of 1 48 was obtained with D12S96 which is located about 5 cM proximal to the keratin gene cluster As is shown for IBS, IE is indicated to be due to a defect in a gene in the region of the Col2A1 gene Also for IE the defect may be in a keratin gene

The markers D17S250 and D17S579, flanking KRT10, both recombine with IE in the persons III 5 and V 1 (Figure 4) Accordingly, haplotype analysis reveals that the interval of D17S250-KRT10-D17S579 is excluded from linkage with IE, which is reflected in a maximal lod score of -4 88 for this interval obtained by multipoint analysis The positive lodscore obtained with KRT10 is due to the fact that the recombinations in III 5 and V 1 are not visible because the affected parent of those persons is homozygous for KRT10

The results from linkage analysis show that among other genes in the region of the Col2A1 gene, members of the type II keratin gene cluster are candidates for both IBS and IE So far, mutations found in keratin genes were in regions coding for the evolutionary conserved domains of the proteins i e (1) the helix initiation peptide at the end of helix domain 1A, (2) the helix termination peptide at the end of helix 2B, (3) the H1 subdomain of type II keratins and (4) the L12 linker region [3 4 and references therein] Sequence analysis gave no indications for mutations in the H1 region, the 1A rod domain, the L12 linker region and the helix termination peptide of K1 in the present families However, a mutation outside the highly conserved parts of K1 may be even more likely regarding the mild phenotypes of IBS and IE [2 24 25] On the other hand, the genetic defect may be present in the K2e gene which is expressed suprabasally in the epidermis from the third or fourth cell layer onwards [26] This expression pattern exactly coincides with the occurrence of the

lesions in patients with IBS and IE i.e. in the upper part of the spinous layer and the granular layer. Mutation analysis in the K2e gene in patients of the present families are underway.

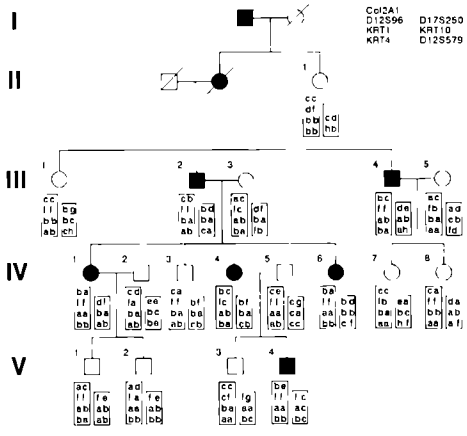


**Fig. 3.** Pedigree of the family with ichthyosis bullosa of Siemens. There is co-segregation with the markers of chromosome 12 (left block) and recombiantion with the markers of chromosome 17 (right block).

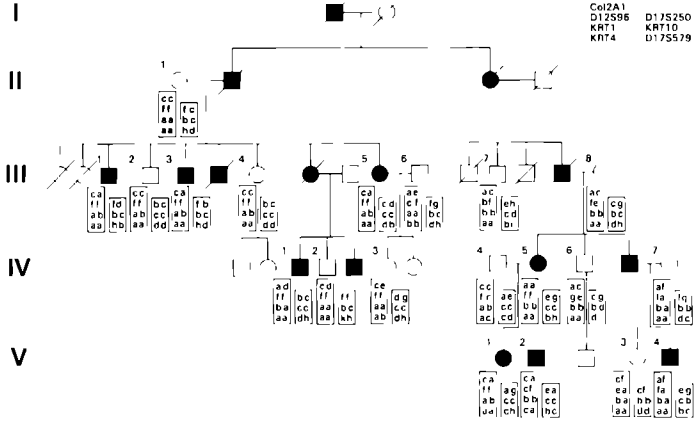


**Fig. 4.** Pedigree of the family with autosomal dominant ichthyosis exfoliativa. Co-segregation can be seen with the marker of chromosome 12 (left block) and recombination with the markers of chromosome 17 (right block)

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# Chapter 5

# Ichthyosis Bullosa of Siemens is Caused by Mutations in the Keratin 2e Gene

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## ABSTRACT

Ichthyosis bullosa of Siemens is a blistering disorder with autosomal dominant inheritance. The disease resembles bullous congenital ichthyosiform erythroderma but is less severe. Keratins K1 and K10 have been implicated in bullous congenital ichthyosiform erythroderma. Linkage analysis pointed to the involvement of a keratin type II gene (12q11-13) in ichthyosis bullosa of Siemens. Mutations in the highly conserved regions of K1, a member of the type II gene cluster, were excluded. The gene coding for keratin 2e is also located in the type II gene cluster and the expression of the gene coincides with the occurrence of epidermolytic hyperkeratosis. Sequence analysis revealed the presence of mutations in the K2e gene in patients with ichthyosis bullosa of Siemens. Three different mutations were detected, one in the 1A domain and two in the 2B domain of the rod. Furthermore, histological and ultrastructural examination of skin biopsies indicated that ichthyosis exfoliativa is identical to ichthyosis bullosa of Siemens. This was confirmed by the results of the molecular analysis. In the family diagnosed as ichthyosis exfoliativa, a mutation was detected which was identical to the mutation found in one of the families with ichthyosis bullosa of Siemens.

## INTRODUCTION

Patients with bullous congenital ichthyosiform erythroderma (BCIE), often referred to as epidermolytic hyperkeratosis, suffer from fragility of the differentiating keratinocytes in the suprabasal layers of the skin. BCIE is caused by mutations in the genes coding for keratin 1 or keratin 10 which are expressed in the suprabasal cells [1-4].

Ichthyosis bullosa of Siemens (IBS) has been described as an entity distinct from BCIE [5-7]. In the former disease hyperkeratosis and blistering are milder than in BCIE. Furthermore, IBS can be differentiated from BCIE by the absence of congenital erythroderma. Light- and electronmicroscopical examination show the features of epidermolytic hyperkeratosis, confined to the stratum granulosum and the upper part of the stratum spinosum, whereas in BCIE these findings are present in the whole suprabasal compartment. Although BCIE and IBS have been described as two diseases, it has also been hypothesized that they are allelic disorders [7,8].

Autosomal dominant ichthyosis exfoliativa (IE) is a recently described type of bullous ichthyosis [9]. Only one family has been published sofar (family 2 in the present study). The clinical symptoms of IE are very similar to IBS. However, according to the original paper, signs of epidermolytic hyperkeratosis were absent.

Linkage analysis in two families with IBS and the family with IE already suggested that both disorders are due to a defect in a member of the type II keratin gene cluster (K1-K8) on chromosome 12q11-13[1]. K1 is a good candidate because mutations in the gene can cause BCIE. However, sequence analysis gave no indications for mutations in the

[1]Steijlen PM, Kremer H, Vakilzadeh F, Happle R, Lavrijsen SPM, Ropers H-H, Mariman LCM Genetic linkage of the keratin type II gene cluster with ichthyosis bullosa of Siemens and with autosomal dominant ichthyosis exfoliativa J Invest Dermatol 1994 103 286-289

conserved regions the H1 region of the head domain of the type II keratins, the helix initiation peptide of the 1A rod domain, the helix termination peptide of the 2B rod domain, the L12 linker between the 1B and 2A rod domains, and an area close to the stutter in the 2B helix The mutations causing BCIE are exclusively detected in these regions of K1 and K10 [1-3,10-12]

The keratin 2e gene is expressed in the upper spinous layers, from the third or fourth cell layer onwards [13] Only these layers show epidermolytic hyperkeratosis in IBS patients [5-7] Sequence analysis of the K2e gene in IBS patients of three different families and in patients of the family with IE revealed three different mutations either in the helix initiation peptide or in the helix termination peptide As was suggested by the clinical picture, IE and IBS are identical disorders

## MATERIALS AND METHODS

*Patients*

Four families were included in the analysis

Family 1 has been previously reported [7] as suffering from IBS

The affected individuals had brownish wrinkled hyperkeratosis and superficial blistering since early childhood Blistering was more pronounced during hot and humid weather and could be provoked by mild trauma Erythroderma had never been present in any of the affected individuals Skin lesions were localized especially on the extensor surfaces of arms and legs and around the umbilicus, knees and ankles In the hyperkeratotic regions superficially denuded areas were observable Occasionally, fresh blisters ranging in size form 0 5 to 2 cm appeared In contrast to patients of the families 2-4, two affected family members also suffered from chronic relapsing pustular eruptions, surrounded by an erythematous flare Light microscopic examination revealed epidermolytic hyperkeratosis limited to the upper part of the epidermis On ultrastructural examination the cells of the granular and upper spinous layer showed marked oedema, while keratohyaline granules were coarse Keratinocytes in the upper spinous layer displayed aggregates of tonofilaments forming V shapes or shells around the nuclei

Family 2 has been reported previously as ichthyosis exfoliativa [9]

We re-examined the family including histological and ultrastructural analyses The patients showed dark grey hyperkeratotic lesions with denuded areas Superficial blistering occurred spontaneously especially during the summer, but occurred also after trivial trauma There was no history of erythroderma in any of the patients Light- and electronmicroscopical examination of a hyperkeratotic area showed the features of epidermolytic hyperkeratosis limited to the granular and upper spinous layers The features consist of keratohyaline clumps, perinuclear vacuolisation and aggregates of tonofilaments forming perinuclear shells (Figure 1a-b) Histopathological examination of a developing blister showed marked extra- and intracellular oedema limited to the keratinocytes of the upper spinous layer PAS-positive deposits were seen within the subcorneal split The number of tonofilaments and keratohyaline granules were reduced The few tonofilament

bundles were localized at the periphery. Tonofilament clumping could be observed focally in the upper spinous layer (Figure 1c).

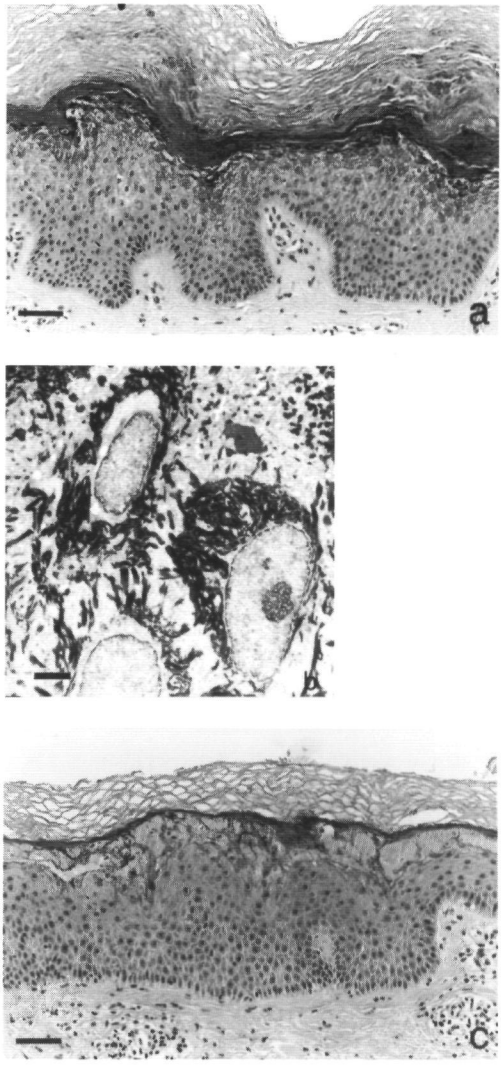


**Fig. 1.** Skin biopsy of a hyperkeratotic lesions in a patient form family 2. **a.** The epidermis is acanthotic and orthohyperkeratotic. In the stratum granulosum and the upper part of the stratum spinosum perinuclear halos and keratohyaline clumps are observed. Scale bar is 50 µ. **b.** Ultrastructurally keratinocytes of the upper spinous layer displaced pronounced aggregates of tonofilaments forming shells around the nuclei. Scale bar, 1 µ. **c.** Skin biopsy of a developing blister in a patient from family 2. The granular and upper spinous layer show a marked extra- and intracellular oedema. Serious deposits were seen within the subcorneal split reflecting early blistering. Clumping of tonofilaments and coarse keratohyaline granules are less obvious. Scale bar, 50 µ.

From family 3 four affected individuals were examined. They showed similar skin lesions as the patients of family 1 and 2. The light and electronmicroscopical findings in all patients were the same as in family 1 and 2. The diagnosis of the disease was IBS.

From family 4 a 21-year-old man had blistering since the age of 3 years. Erythroderma was never present. He suffered from the same skin lesions as the patients of the families

1, 2 and 3. Histological examination showed the features of epidermolytic hyperkeratosis limited to the upper part of the spinous layer. The other family members could not be examined.

*Genomic DNA*

The genomic DNA, used for amplification of the fragments of the K2e gene, was isolated from peripheral blood according to the method described in [14].

*Amplification of DNA fragments*

A fragment of 1.8 kb including the region coding for the H1 and 1A domain of K2e, was amplified with primers 5'-CTGTGACTTTCCTCCCTGGA-3' (sense) and 5'-TCTGCA-GTGAGCCCATCCAGA-3' (antisense). The PCR was performed in a volume of 50 µl containing 0.5-1 µg of genomic DNA, 250 ng of each of the primers, 200 µM of dATP, dGTP, dCTP and dTTP, 10 mM Tris/HCl pH 8.5, 50 mM KCl, 1 mM $MgCl_2$, 0.01% gelatine, 10% dimethyl sulfoxide and 0.5U Taq Polymerase (Perkin Elmer). A PCR program with 35 cycles of 30 sec at 94°C, 1 min at 60°C and 2 min at 72°C was followed. The fragment including the region coding for the C-terminal part of the 2B domain was amplified with the primers 5'-GCAGTGTAAGAATGTGCAAGATG-3' (sense) and 5'-CAGTCACATTGCTGCTGAGGT-3' (antisense). The reaction conditions were as described above with the exception of an annealing temperature of 55°C instead of 60°C. Primer sequences were deduced from the cDNA sequence reported in [13].

*Cycle sequencing*

Purification of the PCR fragments and cycle sequencing were performed as described by.[15] Sequencing of the 1A fragment was performed with the primer 5'-GGTGGCTTTGGGCC-TGGA-3'and of the 2B fragment with the sense primer used for the amplification reaction.

*Allele-specific PCR*

The allele-specific PCR to detect for the transversion of A to C in the 1A domain (family 1) was performed with the sense primer used for amplification of the 1A domain (see above) and the antisense primer 5'-TTGTTGTTGAGAGTTTTGATCG-3'. The last nucleotide at the 3' end of the primer corresponds to the mutation. The conditions for the amplification were as described above with an annealing temperature of 62°C instead of 60°C and 2 mM $MgCl_2$.

*Screening for the mutation present in families 2 and 3*

Screening for the G to A mutation in families 2 and 3 was performed through amplification of a 163 bp fragment with the primer 5'-GTTGAATGACCTGGAGGAGG-3' (sense) and the primer 5'-TTCCCAGTGCCCACACCTG-3' (antisense, intron VII). The latter primer deviates in one nucleotide (underlined) from the sequence of the intron. This destroys an MnlI restriction site which simplifies the analysis. For amplification, the intron

primer was end-labelled with $^{32}P$ The PCR was performed in a reaction mixture of 25 µl with the components in the same concentration as described above Amplification involved 30 cycles of 30 sec at 94°C, 1 min at 55°C and 1 min at 72°C For the digestion with MnlI 10x restriction buffer (supplied by the manufacturer) was added to the endconcentration of 1x and 100 mM spermidine was added to give a concentration of 1mM After the addition of 1U MnlI (New England Biolabs) the reaction mixture was incubated at 37°C for 4 hours The fragments were separated on an 8% nondenaturing polyacrylamide gel

*Screening for the mutation detected in family 4*

The transition of T to C detected in the patient of family 4 creates a HpaII restriction site The testing for the presence of the HpaII site was performed after amplification of the surrounding region with the primers 5'-GTTGAATGACCTGGAGG-3' (sense) and 5'-TCCTCCCTTCCCAGTGCCC-3' (antisense, intron VII) For the amplification the protocol was identical to that described in 'amplification of DNA fragments' The digestion with 2U HpaII was performed as described for the digestion with MnlI Fragments were analyzed on a 2 5% agarose gel

*Prediction of protein structure*

The Chou-Fasman algorithm [16] (Caos Camm Center, Nijmegen) was used for the prediction of protein structure

*Linkage analysis*

Two-point linkage analysis was performed with the Mlink program of the Linkage program package (version 5 03, [17]) The gene frequency of IBS was assumed to be 0 000005 The mutations were treated as polymorphisms with a frequency of 0 001

## RESULTS

To screen for the presence of mutations in the keratin 2e gene, we started with the direct sequencing of the regions coding for the helix boundary peptides and the H1 domain from two patients of the families 1, 2, and 3, from the single patient of family 4 and from two unaffected persons

*family 1* A heterozygous transversion of adenosine ($A_{593}$) to cytosine (C) was detected in patients of family 1 This transversion leads to the substitution of a proline for a glutamine at position 187 (numbering according to [13]) of the predicted protein sequence Through allele specific PCR we studied the inheritance of this mutation in the family to substantiate its disease causing nature The mutant allele could be detected in all affected family members and not in the unaffected persons (Figure 2) Linkage analysis between the mutation and the disease gave a maximum lodscore of 3 42 at a recombination fraction of zero The absence of this mutation in 50 control persons indicated that the mutation is not a common polymorphism

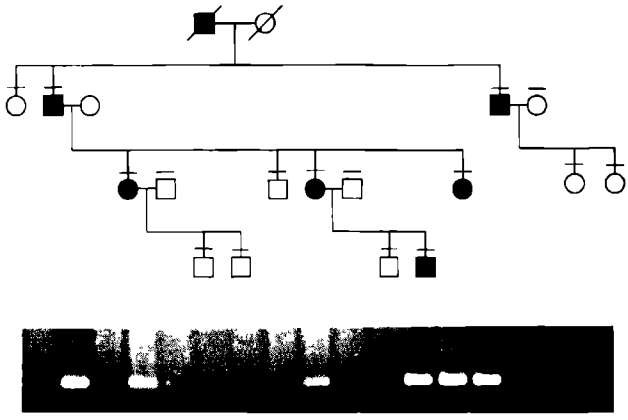

**Fig. 2.** Segregation of the mutation in the K2e gene in family 1. The mutation is detected by allele-specific amplification. In each of the affected persons the mutated allele could be amplified but not in any of the unaffected family members. Persons in the pedigree marked with a bar are included in this analysis.

*family 2 and 3:* In both families 2 and 3 the sequence coding for the helix termination region of the 2B rod domain contained an identical mutation. The GAG codon for Glutamic $acid_{493}$ is changed into an AAG codon leading to the incorporation of a lysine in the predicted K2e protein. The mutation destroyed an MnlI restriction site making it possible to follow the inheritance of the mutation in the families. In family 2 the mutation completely co-segregated with IE (Figure 3a). From family 3 the father was the first affected member and thus a new mutation must have occurred. His three children all were affected and had the mutation (Figure 3b). His parents and 5 brothers and sisters all were unaffected and lacked the mutation. Together with the absence of the nucleotide change in 50 control persons this indicates that the detected mutation causes the disorder. A lod score of 5.46 and 0.54 at no recombination was calculated in two-point linkage analysis between the mutation and IBS in family 2 and 3, respectively.

The mutated $G_{1510}$ is part of a CpG dinucleotide. These are known to have a 42-fold higher mutation rate than expected from random mutation [18]. The same mutation also was detected in a familial IBS patient by McLean et al.[1]

*family 4*: A different mutation was detected in the sequence coding for the helix termination peptide in a familial IBS patient: the transition of $T_{1502}$ to C leads to the substitution of proline for $leucine_{490}$. Although the segregation of the mutation in the family could not be studied because of the lack of blood samples of the relatives, it is likely that this mutation causes the disease, because of the nature of the amino acid substitution (see below) and, because the mutation could not be detected in 50 unaffected persons. A HpaII site is created by the transition.

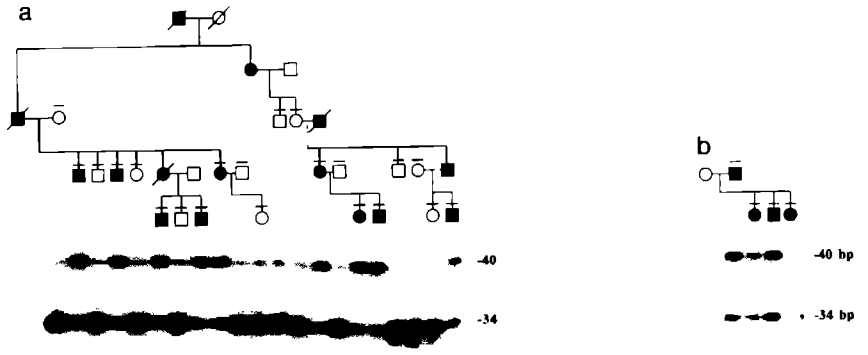


**Fig. 3.** Screening for the $G_{1510}$ to A mutation in family 2 (**a**) and family 3 (**b**) by MnlI digestion of amplified DNA. The mutation destroys a MnlI site resulting in a 40 bp fragment. The normal allele gives a 34 bp fragment. All patients in both families are heterozygous. The unaffected persons are homozygous for the 34 bp fragment. The weak signal of the 40 bp fragment in unaffected relatives probably is the result of incomplete digestion due to the close proximity of the two MnlI sites. The persons included in this study are marked with a bar in the pedigree.

*Nature of the mutations*

In family 1 a nonpolar residue, proline, has been substituted for an uncharged but polar residue, glutamine, of the helix initiation peptide. This means the change of a relatively hydrophillic into a hydrophobic amino acid which can be expected to induce structural changes. Furthermore, proline is known to be interruptive in protein structure. The Chou-Fasman algorithm [16] for prediction of protein structure predicted the mutation to cause a substantial decrease in the length of the α-helix in the corresponding region of the protein.

In both families 2 and 3 the mutation results in the substitution of a basic lysine for the acidic glutamic $acid_{493}$ in the helix termination peptide of the 2B rod domain. The change in charge of the conserved residue can be expected to have a strong effect on the secondary structure of the protein. This is also indicated by the decrease of the length of the helix structure which is predicted by the Chou Fasman algorithm.

Although the substitution of proline for $leucine_{490}$ in family 4 does not result in a change of charge or polarity, the incorporation of proline can be expected to have a disruptive influence (see above). Also this substitution leads to a marked shortening of the α-helix in the predicted protein structure.

## DISCUSSION

Ichthyosis exfoliativa was published as a disorder distinct from IBS on histopathological grounds [9] The identity of the clinical symptoms of both diseases and the linkage to the type II keratin gene cluster induced us to include IE in this study The results of the histological and ultrastructural re-examination of skin biopsies as well as the mutation analysis demonstrated the identity of IBS and IE In the remaining part of the discussion we will only use IBS to indicate the present disorder

Linkage studies already suggested that IBS is caused by a defect in a type II keratin gene located in the gene cluster on chromosome 12q11-13[2] The detection of the mutations in IBS patients finally proved the hypothesis that a defect in keratin K2e causes IBS The mutations show a dominant phenotype as do all but one mutation in keratin genes identified so far [19] In one of the families (family 1) we found a mutation in the helix initiation peptide of the 1A domain In three other families (family 2-4) two different amino acid substitutions were detected in the helix termination peptide of the 2B domain The mutated glutamine$_{187}$ in family 1 and the leucine$_{490}$ substituted in family 4 both are conserved in all type II keratins The mutated glutamic acid$_{493}$ in family 2 and 3 is not only conserved in the type II keratins but in the type I as well These mutations further underline the importance of the integrity of the helix boundary peptides for the appropriate KIF (keratin intermediate filament) formation (cf [10-12,20-23])

The helix boundary peptides are two out of six regions where a close juxtaposition occurs between neighbouring molecules in the KIF and thus specify the correct alignment of these molecules [11,24,25] According to the prediction of the structure of the mutated K2e molecules, the mutations cause a change in the corresponding part of the α-helical rod domain All of the three mutations result in the incorporation of a residue with a lower helix propensity parameter than the normal residues [16] The changes in structure can be expected to affect both the heterodimerization through impairment of the coiled coil formation and the higher order filament assembly, reflected in the disturbed cytoskeleton in keratinocytes of the upper spinous and granular layer of the epidermis (cf [20,22,23,26])

The clarification of the genetic defect causing IBS, finally demonstrates that IBS is distinct from BCIE The divergent expression patterns of K2e and K1 or K10 can explain the clinical and histological differences between both diseases Defects in filament formation are in the skin of IBS patients only visible in the granular layer and the upper spinous layer [5-7,9] This coincides with the expression of the K2e gene in normal skin [13] In BCIE, filament clumping is present in the whole suprabasal compartment which expresses K1 and K10

A substitution of lysine for the glutamic acid corresponding to the Glu$_{493}$ in K2e has been introduced in K14 and subsequently expressed by transient transfection in SCC-13 cells The phenotype is milder than with transfection of a mutated K14 causing the severe EBS-Dowling-Meara in patients [20] This suggests that the nature of the mutations can explain the milder epidermolytic hyperkeratosis of IBS compared to that of BCIE

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# Part III

# Therapy

# Introduction

So far, in monogenic disorders of keratinization treatment is symptomatic Whereas one has to be a splitter to classify these disorders with respect to their origin, to advise families about inheritance and to predict the clinical course, the practical reality of therapeutic intervention is the world of lumpers The process of keratinization constitutes a joint therapeutic target in different disease entities, susceptible to a broad aspecific therapeutic intervention However, as will be shown in chapter 2 and 3 there are some differences in response to oral retinoids Topical treatment of disorders of keratinization include formulations containing urea, lactic acid, propylene glycol and salicylic acid Total body treatment with salicylic acid, however, should be avoided especially in children [1] The introduction of oral synthetic retinoids was a major breakthrough in the treatment of the more severe disorders of keratinization Chapter 1, 2 and 3 describe our experience with these drugs

Since 1962 it is known that topical treatment with vitamin A acid (0,05% all-trans-retinoic acid) is effective in disorders of keratinization[2], however, its toxic side effects limit its use In chapter 4 and 5 the effect of 0,1% 13-cis-retinoic acid is shown in a variety of disorders of keratinization

Calcipotriol is now an established drug in the treatment of psoriasis In a multicenter study the efficacy of calcipotriol ointment was investigated in a large group of disorders of keratinization The results are presented in chapter 6

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# Chapter 1

# Acitretin in the Treatment of Erythrokeratodermia Variabilis

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Dermatologica 1990; 181; 330-333

## ABSTRACT

A patient with erythrokeratodermia variabilis (Mendes da Costa's disease) is presented, and the clinical and histological response to acitretin is described An initial dose of 35 mg of acitretin and a maintenance dose of 25-35 mg resulted in a pronounced and sustained improvement Further reduction of the dosage resulted in a relapse within a few days At the histological level the extensive hyperkeratosis and the moderate dermal inflammatory infiltrate decreased during treatment with acitretin In comparison with the other retinoids available so far, acitretin is the derivative of first choice in the treatment of erythrokeratodermia variabilis Mendes da Costa

Erythrokeratodermia variabilis (EKV) was described by Mendes da Costa in 1925 [1] It is a rare genodermatosis with an autosomal dominant mode of inheritance [2] In 1984 a genetic linkage between EKV and Rh locus has been demonstrated [3] The skin manifestations are highly characteristic Sharply defined figurated patches of erythema that vary in size and localization are observed In addition, sharply demarcated yellow-brown hyperkeratotic plaques of geographic outline are seen, which show a more consistent appearance As a rule, the lesions are distributed symmetrically EKV usually becomes manifest during the first years of life and has a chronic course In general, the skin manifestations are rather extensive

Histologically, the hyperkeratotic plaques are characterized by a laminated orthohyperkeratosis, acanthosis and papillomatosis with elongated capillaries, and a mild mononuclear infiltrate in the perivascular regions [4,5]

Patients with EKV have been treated with several oral retinoids all-trans-retinoic acid[6], 13-cis-retinoic acid[5] and etretinate [7,12] Etretinate is preferred in the treatment of disorders of keratinization for reason of a better ratio of clinical efficacy and side effects [13,15]

This communication reports the clinical and histological changes observed in a patient with EKV during treatment with acitretin

## CASE REPORT

*Clinical aspects*

A 17-year-old woman suffered from a widespread ichthyotic and erythematous skin disorder present since birth During the first year of life she had a generalized erythema, and after 9 months she developed erythematous patches and hyperkeratotic plaques with some itching The lesions varied as to size and localization, stress and heat elicited aggravation and extension of the erythematous lesions No other family member had these skin lesions The patient had been treated with emollients and keratolytics Between 1983 and 1986 she has been treated with etretinate (average dose 25 mg per day) Between 1986 and 1988, 13-cis-retinoic acid was prescribed Etretinate resulted in a considerable improvement, in particular of the hyperkeratotic plaques, whereas 13-cis-retinoic acid at a dosage of 20 mg daily resulted in only a modest improvement She had been without

systemic treatment during 3 months when she presented for the first time at our department. Apart from oral contraception she had no systemic medication.

On examination she had sharply demarcated figurated erythematous lesions symmetrically distributed predominantly on the trunk (Figure 1a). The erythematous patches varied in size and shape within hours. In addition, brownish hyperkeratotic plaques were seen, again sharply demarcated and distributed symmetrically, localized predominantly on the trunk, the knees, hands, elbows and lower legs. The palms showed a moderate keratoderma. Before treatment, blood parameters (clinical chemistry) were within the normal range, and X-ray examination of the vertebral column did not reveal abnormalities.

Apart from bland emollients the patient did not use any topical therapy. Treatment with acitretin was started at a dosage of 35 mg per day, and the hyperkeratotic lesions improved markedly, whereas the erythematous component only showed a moderate improvement (Figure 1).

The dose was adjusted according to the clinical response. During the first 6 months doses below 35 mg per day resulted in a relapse within a few days (Figure 2). After 9 months of treatment the dose could be decreased to 25 mg/35 mg on alternate days, and after 14 months the dose was decreased to 25 mg per day without any aggravation of the condition of the skin. Side effects were limited to moderately dry lips. Blood investigations, urinalysis and X-ray examination during treatment did not reveal abnormalities.

**Fig. 1.** Erythematous and hyperkeratotic lesions in a patient with EKV before treatment **(a)** and after 1 month of treatment **(b)** with acitretin.

*Histopathological Examination*

Figure 3 illustrates the histological observations of a hyperkeratotic lesions at the right lower leg before and after 3 months of treatment with acitretin.

Before treatment, the histological picture was compatible with the diagnosis of EKV. The stratum corneum showed a hyperkeratosis with a broadened stratum granulosum. The

epidermis was acanthotic; however, no pronounced papillomatosis was present. A moderate perivascular infiltrate of mononuclear cells was seen.

After 3 months of treatment the hyperkeratosis and the epidermal acanthosis had diminished considerably. The inflammatory changes in the dermis had only slightly improved.

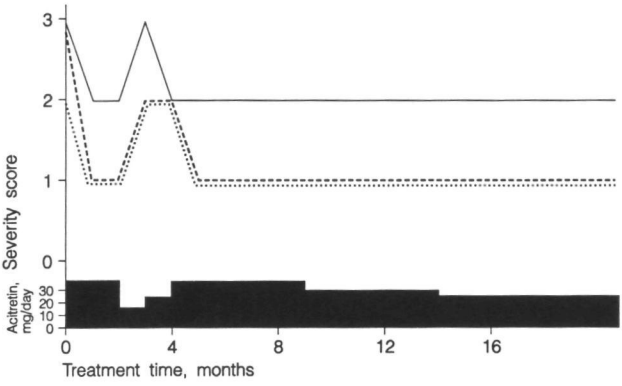


**Fig. 2.** Severity scores in a patient with EKV during treatment with acitretin ———— = Erythema; ------= scaling; .....= palmoplantar keratoderma. Severity score: 1=mild; 2=moderate; 3=severe.

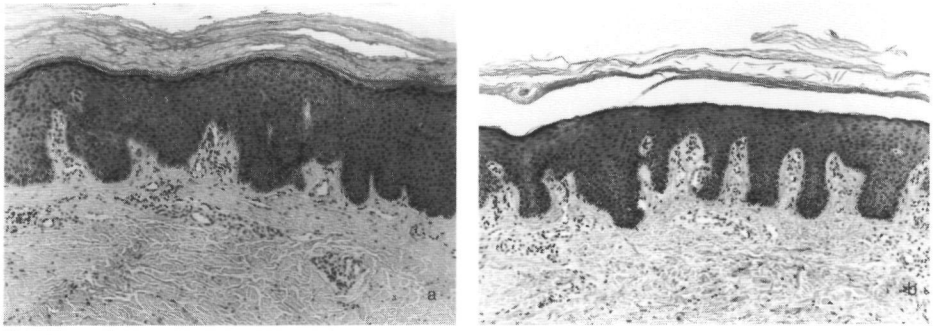

**Fig. 3.** Histological picture of a hyperkeratotic plaque in a patient with EKV before treatment **(a)** and after 3 months of treatment **(b)** with acitretin. x150.

## DISCUSSION

The present observation confirms the effectiveness of retinoids in EKV. The doses of various retinoids and their efficacy in the treatment of EKV are summarized in Table I. The initial dose of acitretin in our case is relatively low, the maintenance dose, however,

**Table I. The effect of retinoids in EKV**

| Retinoid | No of patients | Initial dose | Maintenance dose | Clinical response | Reference No |
|---|---|---|---|---|---|
| Vitamin A | 1 | 50 000 U | - | moderate improvement | 12 |
| All-trans-retinoid acid | 2 | 0-40 mg | 30 mg | nearly clearance | 6 |
| 13-cis-retinoic acid | 1 | 80 mg | - | nearly clearance, erythematous patches recurred | 5 |
| Etretinate | 1 | 50 mg | 25 mg | hyperkeratosis cleared, erythematous patches remained | 7 |
| Etretinate | 1 | 75 mg | 50-75 mg | nearly clearance of hyperkeratosis, erythematous patches remained | |
| Etretinate | 1 | 50 mg | 35 mg | marked improvement of hyperkeratosis | 9 |
| Etretinate | 10 | 0 5-0 9 mg/kg | 0 1-0 6 mg/kg | 90-100% clearance of hyperkeratosis, improvement of erythematous patches | 10 |
| Etretinate | 3 | 1 mg/kg | 0 5 mg/kg | excellent | 11 |
| Etretinate | 1 | ? alternate days | 20 mg/10 mg on | markedly improved | 12 |
| Acitretin | 1 | 35 mg | 25-25 mg | marked improvement of hyperkeratosis, moderate improvement of erythematous lesions | present communication |

is in the dose range of etretinate, as reported by several groups. In other monogenic disorders of keratinization (Darier's disease and X-linked recessive ichthyosis) the effective dose range of acitretin was found to be lower as compared to etretinate.[16 18] In psoriasis, however, the average daily doses of acitretin and etretinate were similar[19] or only slightly lower.[20]

A remarkable observation was the quick relapse following dose reduction after 8 weeks of treatment. Again, the direct dose-response relationship is in line with the experience in Darier's disease.[16] In a group of 6 patients with palmoplantar pustulosis the relapse period following discontinuation of acitretin has been reported to be considerably shorter as compared to etretinate.[21]

Abnormal epidermal differentiation and inflammation are pathogenetic factors in EKV. Retinoids have been shown to interfere with the differentiation process and with various aspects of cutaneous inflammation.[22] In the present case, the hyperkeratosis improved markedly, whereas the erythema diminished to a lesser extent. However, both phenomena improved and relapsed simultaneously following increase and decrease of the dose (Figure 2) The keratinization process is characterized by a decreased number of keratinosomes and abnormal perinuclear aggregation of tonofilaments.[4 5]

The spectrum of diseases responsive to acitretin is comparable with that of etretinate.[23] The relatively short elimination half-life of acitretin clearly has an important impact for the treatment of women of childbearing age, as the period of potential teratogenicity following discontinuation of acitretin is limited to 2 months only Moreover, evidence is accumulating that acitretin has a more direct dose-response relationship in comparison with etretinate.

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# Chapter 2

# Ichthyosis Bullosa of Siemens Responds Well to Low-Dosage Oral Retinoids

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## SUMMARY

Two patients with ichthyosis bullosa of Siemens (IBS) and one patient with bullous ichthyosiform erythroderma of Brocq (BIE) were treated with etretinate Two additional patients with IBS received acitretin All patients had a marked improvement when on retinoids and the maintenance dose required was for IBS 10-25mg per day The patient with BIE was on a maintenance dose of 40-60 mg per day

Bullous ichthyosisform erythroderma is a relatively rare but severe and incapacitating type of congenital ichthyosis This type of epidermolytic hyperkeratosis has been described by Brocq[1] and further delineated by Lapiere[2] However, in 1937 Siemens distinguished a particular type of congenital bullous ichthyosis without erythroderma[3] Over the years this type of ichthyosis has been described as having a relatively mild involvement of the skin and the epidermolytic hyperkeratosis has been restricted to the upper part of the epidermis[4,5]

Aromatic retinoids have been effective in the treatment of various forms of congenital ichthyosis, [6-8] but the treatment of bullous ichthyosis erythroderma of Brocq with oral retinoids has been difficult [9-12] Although a marked reduction of the hyperkeratosis can be achieved, exacerbation of the blistering is a serious complication and often limits the dose of the drug As yet there have been no reports of the use of retinoids in the treatment of ichthyosis bullosa of Siemens (IBS) This present study was to compare the responses of these two types of bullous ichthyosis to therapy with oral retinoids

## CASE REPORTS

The clinical and histological aspects of Case 1 to 4 have been previously described[5] All the patients were monitored at 4-weekly intervals while on treatment

*Case 1*

A 27-year-old woman weighing 63 kg had superficial blistering of the skin on the extensor surfaces of the arms and legs in addition to ichthyosis (Figure 1a) Her father (Case 3), uncle (Case 4), sisters and son were also affected At no time she had an erythroderma A skin biopsy showed mild acanthokeratolysis of the epidermis restricted to the region of the stratum granulosum and upper part of the stratum spinosum

The patient was given acitretin in a dosage of 35 mg daily and after 4 weeks there was marked reduction of the scaling that was virtually absent after 8 weeks of therapy The dose was reduced after 12 weeks of treatment to 30 mg and further reduced after 16 weeks to 25 mg (Figure 1b) The dosage of acitretin was again reduced at 20 weeks because of some hair loss and slight elevation of the liver transaminases A further reduction of the dose was at 24 weeks when she was on 10 and 20 mg on alternate days During the follow-up period of 95 weeks no blistering of the skin had occurred

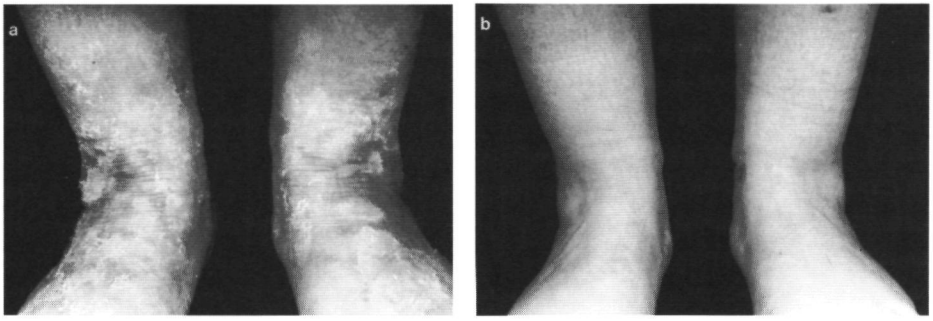


**Fig. 1.** Ichthyosis bullosa of Siemens: **a.** Before treatment; **b.** After 16 weeks of treatment with acitretin (25 mg/day).

*Case 2*

A 21-year-old woman weighing 77 kg presented with the same degree of involvement as seen in Case 1. The patient was given acitretin in a dosage of 35 mg daily and the hyperkeratosis diminished and was virtually absent after 8 weeks of therapy. The dosage was reduced to 30 mg and an excellent response was maintained from 13 weeks with a dosage of 25 mg. During the follow-up period of 22 months no blistering of the skin has occurred.

*Case 3*

The 51-year-old father of Cases 1 and 2 and weighing 77 kg had suffered from a scaly skin since birth. From the age of 15 he also had chronic relapsing pustular eruptions and a biopsy showed a subcorneal blister that was filled with numerous polymorphonuclear leukocytes. He was given etretinate in a dosage of 35 mg daily and after 4 weeks the skin had improved and there was no further pustulation. After 8 weeks of treatment the hyperkeratosis was hardly noticeable and the dosage was further reduced to 25 mg per day. In the 22nd week the dosage was further reduced to 25 mg on alternate days and by the 28th week to 10 mg daily. There was mild scaling of the arms but over the observation period of 14 months there was no blistering or pustulation of the skin.

*Case 4*

A 40-year-old uncle of Case 1 weighing 65 kg had the same disorder with relapsing pustular eruptions and was given etretinate in a dosage of 35 mg daily. After 4 weeks of treatment the hyperkeratosis and pustulation had resolved and the dosage of the retinoid was reduced to 25 mg daily. A further reduction to 10 mg daily resulted in an exacerbation with pustulation and scaling. Over a follow-up period of 12 months a good response was maintained with him having 10 and 20 mg of etretinate on alternate days.

*Case 5*

A 15-year-old male weighing 67 had severe hyperkeratotic (Figure 2a) that covered the entire surface of the body apart from the face, palms and soles. The condition had been present since birth, but because the patient was an orphan it was uncertain about his first year of life and a family history was not available. A skin biopsy from the left forearm showed features of an epidermolytic hyperkeratosis that involved the entire epidermis.

He was treated with etretinate in a dosage of 75 mg daily and within 4 weeks there was a marked improvement. However, a skin biopsy showed even more pronounced histological features despite the clinical improvement. During the subsequent 6 months he was treated with etretinate in a dosage of between 40-60 mg daily and apart from slight recurrence of mild hyperkeratosis with bulla formation he was otherwise satisfactory (Figure 2b).


**Fig. 2.** Bullous ichtyosiform erythroderma of Brocq: **a.** Before treatment; **b.** After 10 weeks of treatment with etretinate (50 mg/day). Note the appearance of some blisters.

## DISCUSSION

Ichthyosis Bullosa of Siemens is a relatively mild condition as compared to bullous ichthyosiform erythroderma. However, the extent of the lesions and the discomfort caused by scaling and pustulation in our cases justified the administration of oral retinoids. As yet there are no other reports on the response of this disorder to systemic retinoids. None of our patients showed any sign of blistering

of the skin during the follow-up period of 12-95 weeks The maintenance dose was much lower than that required for bullous ichthyosiform erythroderma

The efficacy of etretinate in the treatment of BIE is shown in our Case 5, though the maintenance dose of the drug was high at 40-60 mg daily, A maximum dose of retinoids is limited by introduction of blisters in this disorder[9] and it has also been reported that the blister formation was not inhibited by therapy with etretinate[11] as we found in our case

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# Chapter 3

# Acitretin in the Treatment of Lamellar Ichthyosis

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Br J Dermatol 1994; 130: 211-214

## SUMMARY

Etretinate and its metabolite acitretin have been shown to be highly effective in the treatment of various disorders of keratinization, including lamellar ichthyosis. The aim of the present study was to provide further information on acitretin dosage regimens in the management of acitretin in lamellar ichthyosis.

Seven patients with classical manifestations of lamellar ichthyosis participated in the study. Five patients improved markedly, and the remaining patients showed mild to moderate improvement. Two patients improved following gradual incremental increase in dosage (≥35 mg/day). Four patients, including a patient with the erythrodermic variant, required low-dose acitretin treatment (≤25 mg/day), as higher doses resulted in a marked deterioration in their skin condition. The dichotomy with respect to the response to acitretin suggests that lamellar ichthyosis is a spectrum of at least two conditions.

In severe disorders of keratinization retinoids constitute a well-established and highly effective treatment[1,2]. In monogenic disorders of keratinization, however, information on dosage regimens is sparse.

We report the results of acitretin treatment of lamellar ichthyosis. The principal aim of the study was to establish the dose-response relationship in this disorder.

## METHODS

*Patients*

A total of seven patients with lamellar ichthyosis participated in the study. Table I summarizes the patient details. All patients had a history of ichthyosis from birth. Patient 3 had a life-long history of erythroderma. In one patient (patient 7) the family history revealed that two nephews suffered from the same condition. In the other cases family history was negative.

**Table I. Patients details**

| Patients | ♀/♂ | Bodyweigth | Erythroderma | Congenital ichthyosis |
|---|---|---|---|---|
| 1 | ♀ | 40 | | + |
| 2 | ♀ | 39 | | + |
| 3 | ♀ | 59 | + | + |
| 4 | ♂ | 51 | | + |
| 5 | ♀ | 57 | | + |
| 6 | ♂ | 59 | | + |
| 7 | ♂ | 76 | | + |

In six patients the classical picture of non-erythrodermic autosomal recessive lamellar ichthyosis was seen, and in one case (patient 3) erythrodermic autosomal recessive ichthyosis was diagnosed. Flexural involvement, and palmoplantar keratoderma was present in all patients. In patient 1 the condition was associated with almost total alopecia.

Two patients (patients 1 and 3) suffered from pruritus In all patients the severity of the skin condition required systemic treatment with retinoids

In four out of seven patients, treatment was initiated with etretinate, resulting in a moderate to good clinical improvement Apart from mild to moderate cheilitis, no mucocutaneous side-effects occurred in these patients Table II summarizes the results with etretinate In these patients etretinate was discontinued for 1 month before the initiation of acitretin therapy This resulted in a deterioration of skin symptoms during the first 2-3 weeks

**Table II Response to etretinate**

| Patient | Duration (years) | Optimal dose (mg/day) | Improvement | Mucocutaneous side effects |
|---|---|---|---|---|
| 1 | - | - | - | - |
| 2 | 7 | 25 | moderate | mild |
| 3 | 6 | 20 | good | mild |
| 4 | 7 | 25 | good | moderate |
| 5 | - | - | - | - |
| 6 | - | - | - | - |
| 7 | 5 | 50-75 | moderate-good | mild |

*Treatment*

All patients were initially treated with acitetrin 35 mg daily for 4 weeks Thereafter, the dose was individualised on the basis of efficacy and tolerability in each patient Before treatment, and at monthly intervals, blood investigations were carried out ( full blood count, bilirubin, creatinine, urea, uric acid, blood sugar, alkaline phosphatase, transaminases, gamma-glutamyl transpeptidase, cholesterol, triglycerides) Before treatment, after 6 months treatment, and subsequently at yearly intervals, an X-ray of the spine was performed

**Table III. Response to acitretin**

| Patient | Duration (years) | Optimal dose (mg/day) | Improvement | Mucocutaneous side effects |
|---|---|---|---|---|
| **1.** | 4 | 35 | marked | mild |
| **2.** | 4 | 25 | marked | moderate |
| **3.** | 5 | 10-25 | marked | moderate-severe |
| **4.** | 5 | 25 | marked | moderate |
| **5** | ¼ | 35 | mild | severe hairloss |
| **6** | 4 | 10-20 | marked | moderate |
| **7.** | ½ | 55 | moderate | moderate |

*Results*

Acitetrin therapy produced a marked improvement in most patients Table III shows the results in individual patients Figure 1 illustrates the clinical improvement (patient 1) The optimal dose varied considerably between individual patients (Table III) The dynamics of

dosage adjustments, however, provided two distinct response patterns. Some patients improved following each dosage increment (patients 1 and 7: Figure 2).

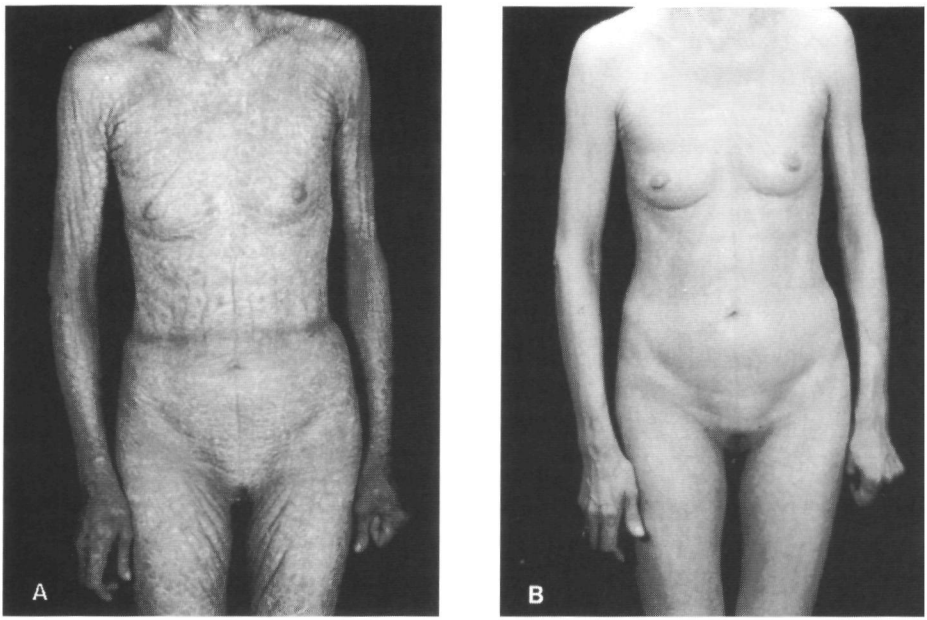
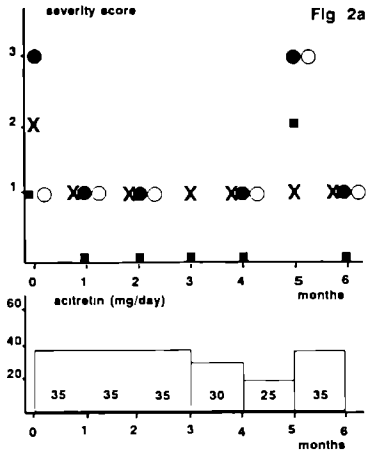



**Fig. 1.** Lamellar ichthyosis before treatment **(a)** and after 6 months treatment **(b)** with acitretin (35 mg/ day, patient 1)

In these patients the maximum dose was limited by mucocutaneous side- effects (cheilitis, epistaxis or hair loss). The remaining patients worsened at a dosage of 35 mg/day, and required lower doses (10-25 mg/day, Figure 3). Reduction of the dosage below 35 mg/day resulted in an improvement of scaling, pruritus and tenderness of the skin. The patient with erythrodermic lamellar ichthyosis (patient 3) showed the most remarkable dose-response relationship in this respect. Figure 2 illustrates dose response relationship of a "high-dose profile" and Figure 3 the dose-response relationship of a "low dose profile". After 4 years treatment there was cosmetically acceptable hair regrowth in patient 1, who had suffered from severe alopecia.

Apart from some degree of cheilitis, epistaxis or hairloss, there were no other side-effects.



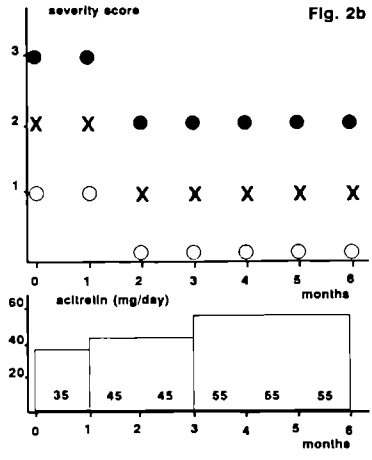
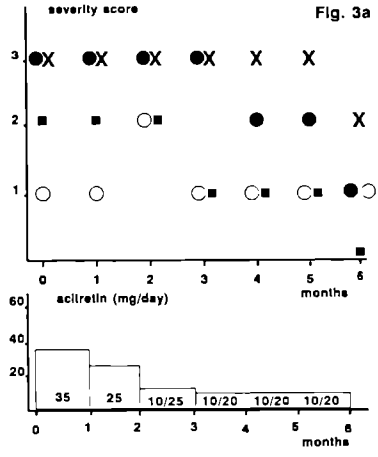




Fig. 2. High dose profile (a) patient 1, (b) patient 7



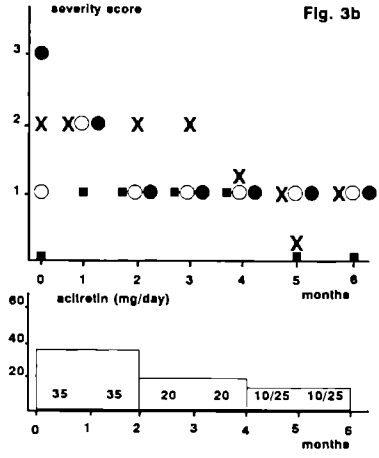
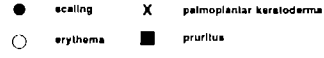




Fig. 3. Low dose profile: (a) patient 3, (b) patient 6




## DISCUSSION

The efficacy of acitretin in the treatment of lamellar ichthyosis has been reported previously[2]. The present study confirms this report, and suggests that two distinct response patterns may occur: (i) a high-dose response, in which maximum efficacy is limited by the tolerance of the individual patient to acitretin; (ii) a low-dose response, in which maximum efficacy is reached at a low dose. In the latter response type, increasing the dose results in more scaling and pruritus. Erythrodermic autosomal recessive lamellar

ichthyosis is characterized by a low-dose response-pattern However, 3 of the patients who responded optimally to the low-dose regimen had clinical and histological features which were similar to those observed in the patients requiring higher doses

The clinical appearance of lamellar ichthyosis is highly variable [3] Patients with lamellar ichthyosis may manifest erythroderma, or they may have the non-erythrodermic variant. In addition, patients may appear non-erythrodermic, but have a previous history of erythroderma At the cellular level, patients with erythrodermic manifestations tend to have increased labelling indices in the epidermis in combination with parakeratosis.[4-6] There is some overlap at the cellular level At the molecular level, patients with erythroderma have increased levels of n-alkanes in the epidermis, in contrast with the normal levels observed in the non-erythrodermic individuals.[7]

It has been suggested that heterogeneity exist within the lamellar ichthyosis group, based on clinical, histological ultrastructural, cellular and biochemical studies The present study demonstrates that the patient with erythrodermic lamellar ichthyosis responded optimally to the low dose regimen The remaining patients who had clinical and histological features of the non-erythrodermic type showed a differential retinoid sensitivity

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# Chapter 4

# Topical Treatment with 13-CIS-Retinoic Acid Improves Darier's Disease and Induces the Expression of a Unique Keratin Pattern

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## SUMMARY

A patient with Darier's disease was treated topically with either 13-cis-retinoic acid 0 1%, all-trans-retinoic acid 0 05%, or their cream base only Both 13-cis-retinoic acid and all-trans-retinoic acid were effective However, all-trans-retinoic acid had to be discontinued because of irritation By contrast, 13-cis-retinoic acid was well tolerated and resulted in a complete remission Both retinoids caused a marked change in the expression of cytokeratins The most remarkable observation was the expression of cytokeratins 4, 13 and 19 at retinoid-treated areas So far known these cytokeratins are absent in adult normal or diseased epidermis and hence provide a cell-biological tool to substantiate a retinoid effect

Darier's disease is an autosomal dominant disorder of keratinization Systemic retinoids have revolutionized the treatment of this chronic distressing dermatosis The results of 13-cis-retinoic acid, etretinate and recently acitretin have been well documented [1-7]

In the older literature topical treatment with all-trans-retinoic acid has been shown to improve disorders of keratinization including Darier's disease [8,9] Irritation of the skin however is a serious drawback of this treatment Therefore the use of topical all-trans-retinoic acid never became an established treatment in Darier's disease and was mainly limited to comedonic and papulopustular acne

The aim of the present study was to investigate 13-cis-retinoic acid as a topical treatment of Darier's disease In particular the question was addressed whether the ratio of therapeutic response and skin irritation was favourable as compared to all-trans-retinoic acid In order to further elucidate the interference of 13-cis-retinoic acid with the process of keratinization and proliferation, an immunohistochemical assessment was carried out

## CASE REPORT

A 62-year-old man suffered from Darier's disease from the age of 42 years At presentation he used no treatment at all Previously he had applied topical steroids and bland emollients during episodes of aggravation Secondary infections of the skin were controlled with antibiotics

Clinical examination of the skin showed an eruption of brownish hyperkeratotic papules, disseminated over the trunk and the upper extremities Characteristic nail changes such as longitudinal bands with a V-shaped nick at the free margin and splitting of the nail plate were observed

Prior to the investigation informed consent was obtained for topical treatment with retinoids Four representative test areas (20 x 20 cm each) localized on the back were selected A shirt with four windows was manufactured permitting accurate and reproducible application of the ointments (Figure 1a)

Using a bilaterally paired comparison, double-blind approach a cream containing 13-cis-retinoic acid in 0 1% concentration the the cream base only (Hoffmann-La Roche, Basel,

Switzerland) were applied in the upper two quadrants. The lower two quadrants were treated with 13-cis-retinoic acid 0.1% and all-trans-retinoic acid (0.05%).
The patient was examined at biweekly intervals. During the first 6 weeks the upper two quadrants were treated twice daily and during the subsequent 6 weeks the creams were applied once a day. The lower quadrants were treated once a day during a period of only 6 weeks.

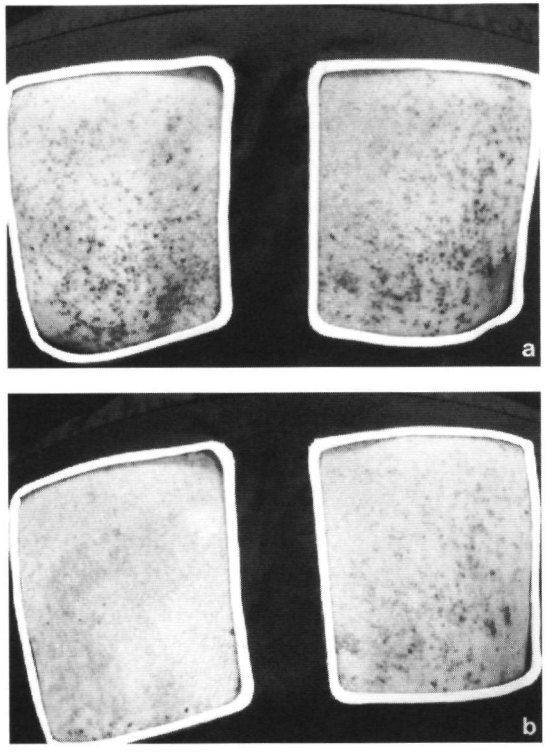


**Fig. 1.** Clinical appearance of untreated and treated skin.
**a.** Upper two test quadrants of untreated lesional skin localized on the back, showing hyperkeratotic papules.
**b.** Total clinical clearance of the 13-cis-retinoic acid treated upper left quadrant after 12 weeks. Slight improvement at the upper right quadrant treated with cream base.

## ANALYTICAL PROCEDURES

*Tissue samples*

Four-millimetre punch biopsies were taken from the upper quadrants before as well as after 6 weeks and 12 weeks of treatment. From the lower quadrants biopsies were taken accordingly before and after 6 weeks of treatment. The biopsies from representative skin lesions were snap frozen in liquid nitrogen and stored at -80°C until processing.

*Antibodies*

Six monoclonal anticytokeratin antibodies and Ki-67, a marker for proliferation[10-12], were used in this study. The specificity and source of the anticytokeratin antibodies are

summarized in Table I. The monoclonal antibody Ki-67 was obtained from Dakopatts, Copenhagen, Denmark.

*Immunohistochemistry*

Four-micronmetre-thick sections were stained with cytokeratin antibodies using an indirect immunoperoxidase staining procedure as described previously.[13] Ki-67 antigen was visualized as described by Gerdes et al.[10,11] with minor modifications.[16]

The percentage of reacting cells in the different cell compartments was estimated using a five-point scale (Figure 2).

**Table I. Specificity of monoclonal cytokeratin antibodies used in this study**

| Antibody | Cytokeratin recognized | Differentiation cell type indicator | Source | Reference |
|---|---|---|---|---|
| 6B10 | CK4 | stratifying, non-cornifying (suprabasal) | Sanbio | 13 |
| RKSE60 | CK10 | stratifying, cornifying (suprabasal) | Euro Diagnostics | 14 |
| 1C7 | CK13 | stratifying, non-cornifying (suprabasal) | Sanbio | 13 |
| 2D7 | CK13 | stratifying, non-cornifying (suprabasal) | Sanbio | 13 |
| RPN 1165 | CK19 | simple epithelia | Amersham | 15 |

## RESULTS

*Clinical response*

After 4 weeks a marked difference was observed between the upper quadrants in favour of the left one which proved to be treated **twice** daily with 13-cis-retinoic acid. Scaling was markedly diminished, papules were flattened, although a slight aggravation of erythema and mild tenderness was experienced. After 6 weeks the area treated with 13-cis-retinoic acid had a normal appearance apart from mild erythema (Figure 1b).

Subsequently the applications were reduced to **once** daily, and this was followed by further improvement and disappearance of the erythema and tenderness. After 12 weeks the experiment was stopped as total clearance was reached in the upper left quadrant treated with 13-cis-retinoic acid.

The upper right quadrant treated with the cream base only showed only a slight improvement during the 12-week period of treatment.

13-Cis-retinoic acid and all-trans-retinoic acid were applied **once** daily to the lower left and right quadrant, respectively. After 6 weeks both areas showed a similar reduction of scaling, and flattening of papules. When compared to the pronounced response of the application **twice** daily of 13-cis-retinoic acid the improvement was moderate. The site treated with all-trans-retinoic acid showed a severe erythema, and the patient experienced burning which necessitated discontinuation of treatment in this area. By contrast, the area treated with 13-cis-retinoic acid showed only a mild erythema.

*Histological findings*

Biopsies taken before and during treatment with the cream base only showed a similar picture. The stratum corneum displayed a marked hyperkeratosis of the ortho- and parakeratotic type. The epidermis demonstrated acanthotic and acantholytic foci with typical dyskeratosis and suprabasal cleft formation. A pronounced band-like mononuclear infiltrate was noted in the papillary dermis.

The sites treated with 13-cis-retinoic acid as well as all-trans-retinoic acid treated showed a marked reduction of the above mentioned abnormalities resulting in a mild acanthosis and a slight perivascular infiltrate.

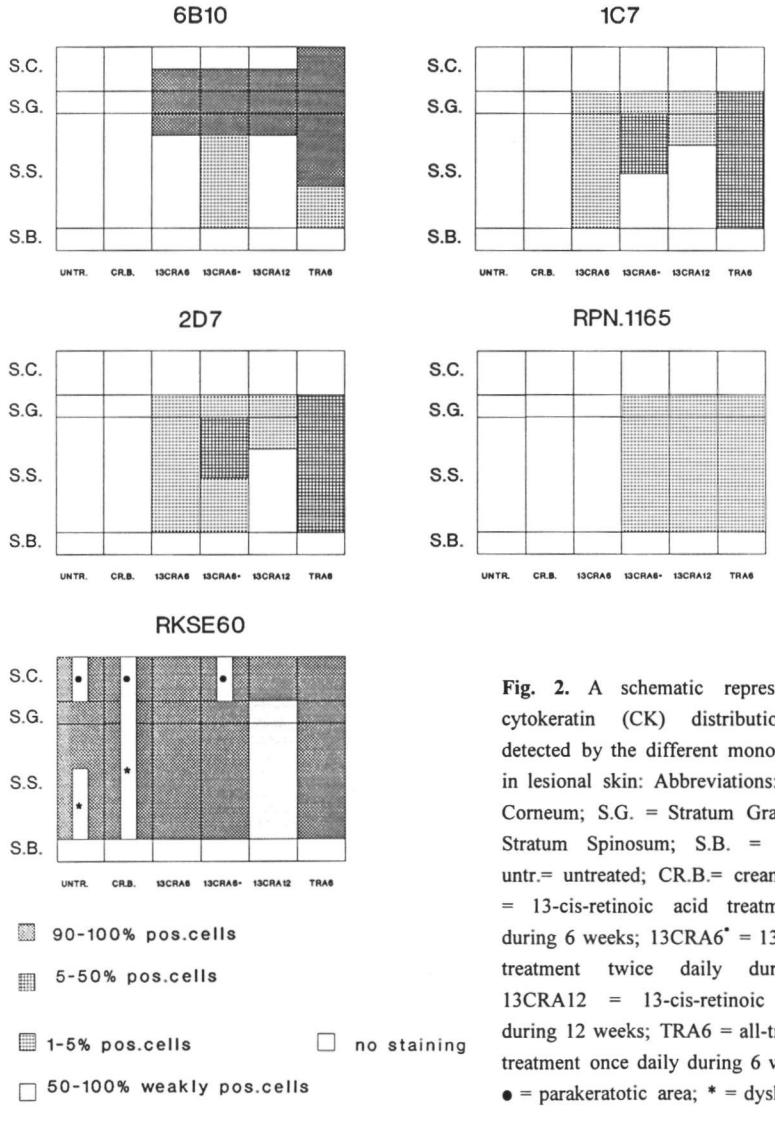


**Fig. 2.** A schematic representation of the cytokeratin (CK) distribution patterns as detected by the different monoclonal antibodies in lesional skin: Abbreviations: S.C. = Stratum Corneum; S.G. = Stratum Granulosum; S.S. = Stratum Spinosum; S.B. = Stratum Basale; untr.= untreated; CR.B.= cream base; 13CRA6 = 13-cis-retinoic acid treatment once daily during 6 weeks; 13CRA6* = 13-cis-retinoic acid treatment twice daily during 6 weeks; 13CRA12 = 13-cis-retinoic acid treatment during 12 weeks; TRA6 = all-trans-retinoic acid treatment once daily during 6 weeks

• = parakeratotic area; * = dyskeratotic area

*Immunohistochemical findings*

The results obtained with the immunoperoxidase technique on frozen sections are summarized in Figure 2.

Cytokeratins 4, 13 and 19 were not expressed in biopsies taken before treatment and taken from the sites treated with the cream base only. Cytokeratin 4 showed expression of varying intensity in the biopsies taken from the sites treated with 13-cis-retinoic acid and all-trans-retinoic acid. Staining was limited to the upper part of stratum spinosum, stratum granulosum and stratum corneum (Figure 3a). Remarkably, staining was more extensive in the biopsy taken from the site treated with all-trans-retinoic acid (Figure 3b).

The monoclonal antibodies 1C7 and 2D7, recognizing cytokeratin 13, showed similar focal staining patterns with varying intensity in biopsies taken from the sites treated with 13-cis-retinoic acid and all-trans-retinoic acid. Positive keratinocytes were found in the stratum spinosum (Figure 3c). In the biopsy from the site treated with all-trans-retinoic acid (Figure 3d) the expression of cytokeratin 13 appeared to be stronger.

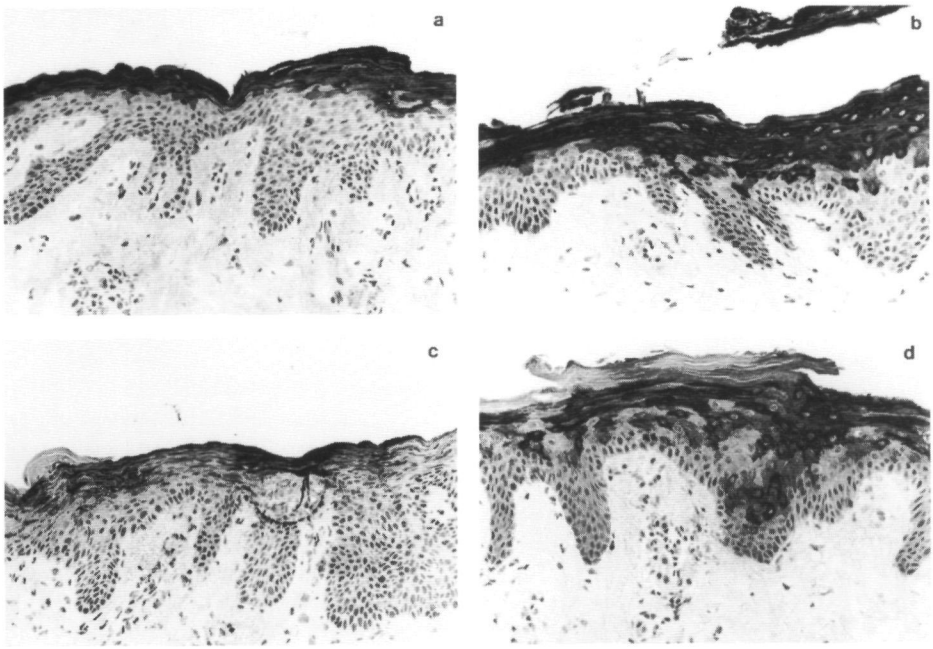


**Fig. 3.** Immunoperoxidase staining patterns of frozen sections from biopsies taken after 12 weeks of treatment. **a.** 13-Cis-retinoic acid treated site, showing cytokeratin 4 expression; **b.** All-trans-retinoic acid treated site, showing cytokeratin 4 expression; **c.** 13-Cis-retinoic acid treated site, showing cytokeratin 13 expression; **d.** All-trans-retinoic acid treated site, showing cytokeratin 13 expression.

Cytokeratin 19 was expressed focally only in the stratum granulosum and stratum spinosum in the biopsies taken from the sites treated with 13-cis-retinoid acid twice daily or with all-trans-retinoic acid (Figure 2).

Cytokeratin 10 was observed in the suprabasal compartment. In the biopsies taken before treatment and from the site treated with cream base only, the acantholytic and dyskeratotic regions showed no staining. The parakeratotic stratum corneum did not show staining either. In the sample taken after 12 weeks of treatment with 13-cis-retinoic acid expression of cytokeratin 10 was observed only focally in the stratum spinosum and stratum granulosum.

Ki-67-positive nuclei were assessed in all biopsies In lesional skin being untreated or treated with the cream base only, the numbers of positive nuclei were 36 and 37 per millimetre section length, respectively. At the sites treated with 13-cis-retinoic acid values were 24, 30 and 58 positive nuclei per millimetre, respectively. At the all-trans-retinoic-acid-treated site the counts were 35 per millimetre.

## DISCUSSION

The present case is the first demonstration of a therapeutic effect of topical 13-cis-retinoic acid in a monogenic disorder of keratinization.

The clinical results of the present study show that topical treatment with 13-cis-retinoic acid and all-trans-retinoic acid in a 0.05% concentration are effective in Darier's disease. The clinical improvement was confirmed at the histopathological level by a marked reduction of acanthosis and hyperkeratosis. Topical treatment with 13-cis-retinoid acid during 12 weeks resulted in a total remission of the treated test area.

Compared to the intolerable tenderness caused by treatment with all-trans-retinoic acid, irritation induced by treatment with 13-cis-retinoic acid once daily was negligible Topical application of 13-cis-retinoic acid with a concentration up to 0.3% showed an excellent tolerance in the hamster model[17]. Therefore the concentration of 13-cis-retinoic acid used in the present study might be increased 2-3 times which might considerably enhance the therapeutic effectivity.

Our results confirm that topical all-trans-retinoic acid is effective in Darier's disease.[9] In the hairless mouse model for ichthyosis a beneficial effect of 13-cis-retinoic acid had a comparable potency as to the reduction of scaling[18]. In view of the observations of Stuttgen[8] with all-trans-retinoic acid, the excellent tolerability of 13-cis-retinoic acid in the animal model[17], the beneficial effect in the hairless mouse model[18] and the present study, 13-cis-retinoic acid may have wider implications as a therapeutic tool in disorders of keratinization other than Darier's disease.

Transformation of 13-cis-retinoic acid into all-trans-retinoic acid has been reported[19,20]. In this respect the effect of ultraviolet radiaton is of significance. In our patient applications were carried out on sites not exposed to sunlight. Nevertheless it is remotely possible that relatively low doses of all-trans-retinoic acid may contribute to the therapeutic effect on

the sites treated with 13-cis-retinoic acid. The clinical effect of low doses of all-trans-retinoic acid remains to be explored.

The cytokeratin pattern of Darier's disease has been reported by Burge et al. [21]. In the present report we can confirm their observations that cytokeratin 10 was not consistently expressed in acantholytic regions and their observation of the absence of cytokeratin 19 in untreated lesional skin. We have extended their observations with respect to the absence of cytokeratin 4 and 13 in untreated skin (Figure 2).

Topical application of 13-cis-retinoic acid and all-trans-retinoic acid induced the expression of cytokeratins 4, 13 and 19 which are not expressed in normal adult epidermis[22]. The expression of these cytokeratins is limited to the suprabasal compartment. To the best of our knowledge, the in vivo expression of these cytokeratins has never been described before, either in normal or diseased epidermis. Expression of cytokeratins 4, 13 and 19 was reported in a differentiation model using cultured foreskin keratinocytes and in 15- and 20-week-old fetal epidermis.[22] In vitro it was shown by Kopan et al.[23] that retinoic acid induced synthesis of cytokeratins 13 and 19. Ponec[24] reported the induction of cytokeratins 4, 13 and 19 in reconstructed epidermis in vitro by all-trans-retinoic acid and acitretin. The selective induction of these keratins, which are found in simple and non-cornifying stratified epithelium, is of practical significance as a marker for retinoid effect. After treatment with both 13-cis-retinoic acid and all-trans-retinoic acid acantholysis and dyskeratosis were no longer present. Twelve weeks of treatment with 13-cis-retinoic acid resulted in a down-modulation of cytokeratin 10, indicated by a rather weak staining pattern (Figure 2).

In a recent study we demonstrated that normal epidermis had a total number of 24.7 ± 2.2 Ki-67-positive nuclei per millimetre section length[12]. Five of the 6 biopsies examined in the present study showed an increased number of Ki-67-positive nuclei corresponding with an increased recruitment of actively cycling epidermal cells. This observation indicates that the increased labelling index as suggested by Lachappelle et al.[25] can be explained at least in part by an escape of cells from the non-dividing basal cell population. The recruitment process of cycling cells has been shown to be a non-specific phenomenon in conditions characterized by a benign hyperproliferation such as response to injury or psoriasis[12]. Therefore, it is attractive to speculate that the increased nuclear staining with Ki-67 in Darier's disease is an attempt to normalize the epidermal disintegration caused by dyskeratosis and acantholysis. In contrast to the marked changes of keratinization induced by topical 13-cis-retinoic acid and all-trans-retinoic acid, these retinoids did not affect cell proliferation.

We conclude that topical treatment with 13-cis-retinoic acid is effective in Darier's disease. The assessment of cytokeratins 4, 13 and 19 may be useful to demonstrate interference with the disease process at the cellular level and may serve as a marker to indicate the bio-availability of the drug.

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# Chapter 5

# Topical Treatment of Ichthyoses and Darier's Disease with 13-CIS-Retinoic Acid. A Clinical and Immunohistochemical Study.

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## SUMMARY

In a bilaterally paired double-blind comparison study, a cream containing 0 1% 13-cis-retinoic acid (13-cis-RA) and cream base only were applied over 4 weeks in seven patients with non-erythrodermic lamellar ichthyosis (NELI), two patients with Darier's disease and one patient with autosomal dominant ichthyosis vulgaris (ADIV) In two patients with NELI and two patients with Darier's disease a half-side effect was observed in favour of the side treated with 13-cis-RA

In three patients an induction of cytokeratin-4, and in one of these patients expression of cytokeratin-13, were observed after therapy Topical 13-cis-RA appears to be a promising approach in the treatment of disorders of keratinization The selective modulation of the cytokeratin pattern may provide an immunohistochemical tool to investigate the mode of action of retinoids

The oral administration of retinoids has revolutionized the treatment of disorders of keratinization [4 6 8 9 17] However, because of the risk of systemic side effects, topical application would be preferable Several reports indicate that topical treatment with all-trans-retinoic acid has a therapeutic effect in disorders of keratinization [1 7 16 29] However, its irritative effect restricts its clinical application to the treatment of acne Recently, a new formulation containing 13-cis-retinoic acid (13-cis-RA) has become available for topical treatment

The aim of the present study was to investigate the clinical effect of topical application of 13-cis-RA in patients with ichthyoses and Darier's disease The question was also addressed as to whether in vivo topical treatment with retinoids modulates the expression of cytokeratins as has been described in vitro [10 19] The expression of cytokeratins was assessed by immunohistochemical methods using a panel of well-characterized monoclonal and polyclonal anti-cytokeratin antibodies

## PATIENTS AND METHODS

*Patients*

Seven patients with non-erythrodermic lamellar ichthyosis (NELI), two patients with Darier's disease and one patient with autosomal dominant ichthyosis vulgaris (ADIV) were treated with topical 13-cis-RA after informed consent was obtained Before the trial, systemic treatment was stopped Further details regarding sex, age and previous treatment are summarized in Table I

In every patient two comparable and representative test areas were selected In this bilaterally paired double-blind comparison study, a cream containing 0 1% 13-cis-RA and cream base only (Hoffmann-La Roche, Basel, Switzerland) were applied over 4 weeks Patients were instructed to apply the creams thinly and evenly twice daily If intolerable irritation occurred, they were advised to reduce the frequency

**TABLE I Clinical data**

| Patient | Sex | Age (years) | Diagnosis | Time from stopping oral retinoid to starting trial | Previous topical treatment |
|---|---|---|---|---|---|
| 1 | m | 40 | NELI | not applicable | petrolatum |
| 2* | m | 34 | NELI | 19 days | propylaenglycol, petrolatum |
| 3 | f | 53 | NELI | 14 months | none |
| 4* | f | 41 | NELI | not applicable | urea |
| 5 | f | 24 | NELI | 22 months | propylaenglycol, urea |
| 6* | m | 33 | NELI | 15 days | acidum lacticum |
| 7* | m | 38 | NELI | 10 years | urea |
| 8 | f | 36 | Darier's disease | 10 years | none |
| 9 | f | 18 | Darier's disease | 12 days | none |
| 10 | m | 42 | ADIV | not applicable | urea |

* patients from one family
NELI = non-erythrodermic lamellar ichthyosis, ADIV = autosomal dominant ichthyosis vulgaris

*Analytical procedures*

*Tissue samples* Biopsies from representative skin lesions within the test areas were taken from every patient, one at the beginning of the trial and one from each area after 4 weeks of treatment The biopsies were snap-frozen in liquid nitrogen and stored at -80° C until processed

*Antibodies* Used in the study were 16 monoclonal and 2 polyclonal anticytokeratin antibodies (Table II) The polyclonal antibodies AF87 and AF124 were a kind gift from Dr Stuart H Yuspa (National Cancer Institute, Bethesda, Maryland, USA) The monospecific antibodies were raised against C-terminal synthetic peptides of cytokeratins 1 and 6 in rabbits as described previously [23] The mouse monoclonal antibody KA12, reacting with cytokeratin 6 on two-dimensional gels from oesophagus and placenta was a kind gift from Dr R Nagle (Tucson, Arizona) This antibody may also react partially with cytokeratin 5 (Nagle, personal communication)

The specificity and references for the other antibodies are summarized in Table II

**TABLE II.**

| Monoclonal/polyclonal antibody | Cytokeratin specificity | Type of differentiation | Reference |
|---|---|---|---|
| AF 87 | 1 | stratifying, cornifying | 23 |
| RKSE 60 | 10 | (suprabasal) | 21 |
| RCK 102 | 5 (+8) | basal cells | 2 |
| RCK 107 | 14 | | 30 |
| AF 124 | 6 | hyperproliferation | 23 |
| KA 12 | 6 (+5) | | |
| 6B10 | 4 | stratifying, non- | 14 |
| 1C7 | 13 | cornifying (suprabasal) | 14 |
| 2D7 | 13 | | 14 |
| RCK 105 | 7 | simple epithelial cells | 21 |
| LE 41 | 8 | | 11 |
| Cam 5.2 | 8 | | 27 |
| M20 | 8 | | 25,26 |
| RGE 53 | 18 | | 20 |
| RCK 106 | 18 | | 21 |
| CK 18 2 | 18 | | 2 |
| 2C8 | 18 | | 25,26 |
| RPN 1165 | 19 | simple epithelia, some stratifying epithelia | 2,12 |

*Immunohistochemistry.* Sections (4μm) were stained with the cytokeratin antibodies using a two-step indirect immunoperoxidase staining procedure as described previously.[30]

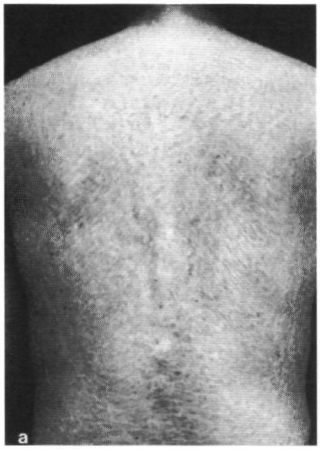
## RESULTS

### *Clinical response*

The clinical results obtained after administration of 13-cis-RA are summarized in Table III. Two of the seven patients with NELI showed a half-side effect in favour of the 13-cis-RA treated side. The clinical improvement in these patients consisted of a marked reduction in scaling and an improvement in the smoothness of the skin (Figure 1). Both patients with Darier's disease showed a moderate reduction of the hyperkeratotic lesions in the retinoid-treated areas. No clinical improvement was observed in the areas treated with cream base only. The patient with ADIV showed no (half-side) effect upon treatment. Both patients showed only a slight reduction of scaling.

Three of the four patients responding to 13-cis-RA showed a marked erythema with itching, tenderness and a burning sensation. After 3 days patient 2 with NELI displayed a marked redness and swelling of the skin which led him to stop the treatment for 1 week. Nevertheless, a marked half-side effect was induced and could be maintained by 2-weekly applications (Figure 1). In contrast to the patients with a marked improvement, five patients without a clinical response showed a mild non-tender erythema. The other non-responding patient (patient 7) complained of an itching redness of the scrotum, which

might have been due to spreading of the retinoid. After 2 weeks patient 6, having been free of systemic therapy for 4 weeks, restarted therapy with acitretin (35 mg /day) because of an unacceptable worsening of his ichthyosis. In this patient the topical applications were continued.



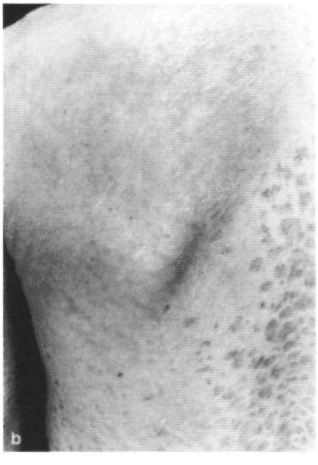





**Fig. 1.** patient 2 with NELI. **a.** clinical appearance of untreated skin; **b.** side treated with topical 13-cis retinoic acid; **c.** side treated with cream base only

*Cytokeratin expression patterns*

The immunohistochemical findings with the cytokeratin antibodies are summarized in Table IV. Before treatment both markers for cornifying epithelia, i.e. AF87 and RKSE60, recognizing keratins 1 and 10, respectively, reacted with the suprabasal compartment with a homogenous pattern. Treatment with topical 13-cis-RA did not change their reaction pattern. The basal cell markers RCK102 and RCK107 stained the basal layer in all biopsies taken before and after treatment. No consistent change in staining patterns could be observed after treatment with 13-cis-RA.

**TABLE III. Clinical responses at 13-cis-RA treated sides (13-cis-RA side) and cream base only treated sides (cream base side) after 4 weeks**

| Pat | Diagnosis | Treated area | Clin resp * cr base side | Clin resp* 13-cis-RA side | Erythema** 13-cis-RA side | Side-effects 13-cis-RA side |
|---|---|---|---|---|---|---|
| 1 | NELI | frontal aspects<br>legs | 1 | 1 | 1 | - |
| 2 | NELI | back | 0 | 3 | 2 | itching<br>swelling |
| 3 | NELI | breast, abdomen | 1 | 0 | 1 | - |
| 4 | NELI | frontal aspects upper legs | 0 | 0 | 1 | - |
| 5 | NELI | upper arms | 0 | 3 | 2 | itching, burning,<br>swelling |
| 6 | NELI | frontal aspects upper legs | 1 | 1 | 0 | - |
| 7 | NELI | frontal aspects upper legs | 1 | 1 | 1 | erythema scrotum |
| 8 | Darier's disease | back<br>lower legs | 0<br>0 | 2<br>0 | 2<br>1 | itching<br>burning |
| 9 | Darier's disease | back | 0 | 2 | 1 | itching |
| 10 | ADIV | frontal aspects<br>upper legs | 1 | 1 | 1 | - |

* Clinical response, 0=none, 1=slight, 2=moderate, 3=marked

** Erythema 0=none, 1=mild, 2=marked

**TABLE IV. Staining patterns of anticytokeratin antibodies before treatment and after 4 weeks of topical application with 13-cis-RA**

| Antibody | CK | Staining pattern | Before treatment Frequency of positive staining cases NELI N=7 | DARIER N=2 | ADIV N=1 | After treatment Frequency of positive staining cases NELI N=7 | DARIER N=2 | ADIV N=1 |
|---|---|---|---|---|---|---|---|---|
| AF 87 | 1 | SB/H | 7 | 2 | 1 | 7 | 2 | 1 |
| RKSE60 | 10 | SB/H | 7 | 2 | 1 | 7 | 2 | 1 |
| RCK102 | 5+8 | B/H, SB/S | 6 | 2 | 1 | 6 | 2 | 1 |
| | | B/H, SB/F | $1^{2*}$ | - | - | $1^{2}$ | - | - |
| RCK107 | 14 | B/H, SB/S | 7 | 2 | 1 | 6 | $1^{8}$ | 1 |
| | | B/H, SB/F | - | - | - | $1^{2}$ | $1^{9}$ | - |
| AF124 | 6 | SB/H | $1^{2}$ | $1^{9}$ | - | - | $1^{9}$ | - |
| | | SB/F | $3^{1\,5\,6}$ | - | 1 | $1^{2}$ | $1^{8}$ | - |
| | | SB/S | $1^{4}$ | $1^{8}$ | 1 | $1^{4}$ | - | - |
| KA12 | 6 (+5) | SB/H | $1^{2}$ | $1^{9}$ | - | - | - | - |
| | | SB/F | $2^{4\,6}$ | $1^{8}$ | 1 | $4^{2\,3\,4\,6}$ | 2 | - |
| | | SB/S | $2^{3\,5}$ | - | - | $2^{1\,7}$ | - | - |
| 6B10 | 4 | SB/S | - | - | - | $2^{2\,4}$ | $1^{8}$ | - |
| 1C7 | 13 | SB/S | - | - | - | - | $1^{8}$ | - |
| 2D7 | 13 | SB/S | - | - | - | - | $1^{8}$ | - |

B = Basal staining; SB= Suprabasal staining, H = Homogenous (90-100%); F = Focal (5-90%), S = Sporadic (0,5-5%);
* Numbers in superscipt correspond with the patients listed in Table I

The two antibodies AF124 and KA12, reactive with hyperproliferation-related keratins, were found to be positive in biopsies taken before and after treatment. No consistent change in the expression pattern was induced by the 4-week topical application of 13-cis-RA. The markers for non-cornifying stratified epithelia, i.e. antibodies 6B10 to cytokeratin 4 and 1C7 and 2D7 to cytokeratin 13, showed no reaction in the biopsies taken before treatment. After treatment with 13-cis-RA sporadic suprabasal keratinocytes reacted with 6B10 in two patients with NELI and one patient with Darier's disease. One patient with Darier's disease showed sporadic staining with 1C7 and 2D7 after topical applications with 13-cis-RA. The markers for simple epithelial cells, i.e. antibodies to cytokeratin 7, 8, 18 and 19 (Table II), showed no epidermal staining in the biopsies taken either before and after treatment.

Treatment with cream base showed no modulation of the cytokeratin reaction patterns.

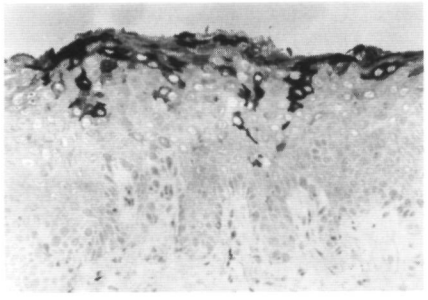


**Fig. 2.** patient 9. Sporadic immunohistochemical staining pattern of frozen section of involved epidermis after treatment with topical 13-cis-RA with the antibody to cytokeratin 4.

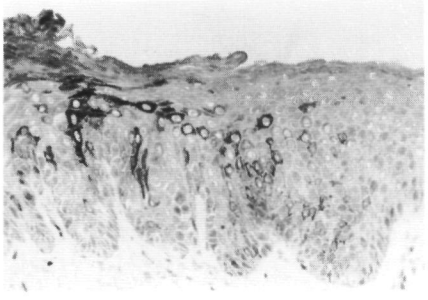


**Fig. 3.** patient 9. Sporadic immunohistochemical staining pattern of frozen section of involved epidermis after treatment with topical 13-cis-RA with the antibody to cytokeratin 13.

## DISCUSSION

After 4 weeks of treatment four out of the ten patients (two NELI and two Darier's disease) showed a marked reduction in scaling and an improvement in smoothness of the skin only at the 13-cis-RA treated side. All these responding patients demonstrated, in addition, an itching erythema, while the remaining six patients, not showing any significant half-side effect, displayed only a mild erythema. Both patients with Darier's disease responded well, which is in accordance with a previous observation.[28] The great inter-individual variation as to responsiveness in patients with NELI is difficult to explain. It is known that 13-cis-RA is unstable to light exposure.[5,13] However, the inter-individual variation in clinical response cannot be explained by differences in exposure to ultraviolet radiation. All patients protected the treated areas from light, so photoinactivation is unlikely as an explanation for the inter-individual variation.

The efficacy of topical treatment with 13-cis-RA may be improved by changing the concentration, the form of application (such as occlusion) and the basis.[18] The clinical

response and penetration are markedly influenced by the base [18] Other retinoids effective in topical application, such as the arotinoids, might be better candidates than 13-cis-RA for the treatment of disorders of keratinization because they cause less irritation [3]

The immunohistochemical examination revealed a selective modulation of the cytokeratin pattern after retinoid therapy The induction of cytokeratin 4 was demonstrated in three out of ten patients after topical application of 13-cis-RA Sporadic expression of cytokeratin 13 was found in one patient after therapy The induction of cytokeratin 4 and/or 13 by retinoids in this study confirms previous in vivo[22 24 28] studies and other in vitro observations [10 19]

The induction of these cytokeratins which are normally found in fetal, but not in adult, epidermis[15], in our view reflects a certain degree of dedifferentiation of the epidermis caused by the retinoid

Further investigations with topical retinoids are needed to optimize the clinical response by adapting the concentration, the base and the mode of application to the individual patient Increasing the length of the treatment period might also improve the response

The modulation of the cytokeratin expression pattern by topical application of 13-cis-RA may provide an immunohistochemical approach to elucidate and to monitor the mode and degree of action of retinoids

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# Chapter 6

# Efficacy, Tolerability and Safety of Calcipotriol Ointment in Disorders of Keratinization: Results of a Randomized, Double-Blind, Vehicle Controlled, Right/Left Comparative Study

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**SYNOPSIS**

To study the effect of topical calcipotriol (calcipotriene) on disorders of keratinization 67 patients were included in a randomized, double-blind, placebo-controlled, right-left comparative study After treatment for 12 weeks, calcipotriol had improved lesions of congenital ichthyosis, X-linked ichthyosis and ichthyosis vulgaris

**ABSTRACT**

**Background and Design:** Disorders of keratinization are a heterogenous group of diseases, which have in common a defect in cornification The bioactive form of vitamin $D_3$ has been shown to modulate epidermal proliferation and differentiation The purpose of the present study was to determine the effect of the synthetic vitamin $D_3$ calcipotriol in a randomized, double-blind, placebo-controlled, right/left comparison comparative study The 67 patients included were at least 12 years of age Nine patients had ichthyosis vulgaris, 8 X-linked ichthyosis, 10 congenital ichthyosis, 20 hereditary palmo-plantar keratoderma, 9 keratosis pilaris and 11 Darier's disease All patients applied calcipotriol ointment 50 μg/g and placebo (vehicle of calcipotriol ointment) twice daily for up to 12 weeks Patients were allowed to use up to 120 g calcipotriol ointment per week

**Results:** At the end of treatment calcipotriol ointment had improved the ichthyoses, although to a variable degree No therapeutic effect was detected in palmo-plantar keratoderma or keratosis pilaris Eight of 11 patients with Darier's disease had to be withdrawn because of skin irritation or a worsening of the disease Skin irritation occurred in 18 cases (26%) only on the calcipotriol treated side, and in 1 case (1%) only on the placebo treated side Nine cases (13%) had irritation on both sides The amount of calcipotriol ointment used per week was lowest in palmo-plantar keratoderma (mean 11 8 g/week, range 2 1-25 6 g/week) and highest in congenital ichthyosis (mean 59 3 g/week, range 11 4-94 7 g/week) There was no clinically significant change of serum calcium levels during the treatment period

**Conclusions:** It is concluded that short-term treatment with calcipotriol ointment 50 μg/g, used in amounts up to about 100 g per week, is moderately efficaceous, well-tolerated and safe in adult patients with various ichthyoses

Disorders of keratinization comprise a heterogeneous group of diseases characterised by dry and scaly skin (1) They include the ichthyoses, Darier's disease, pityriasis rubra pilaris, keratodermas and follicular keratoses The disorders of keratinization are chronic, often inherited, diseases with onset in childhood Treatment is often unsatisfactory, although lubrication with ointments or creams may soften the skin, and topical preparations containing salicylic acid may remove the scaling In some of the disorders, retinoids administered topically, but mainly systemically, are effective (2) Because of the associated risk of severe side-effect, the systemic retinoids are only used in severe and widespread cases

Although the disorders of keratinization are a heterogeneous group of diseases, they have in common a defect in cornification The underlying pathogenic mechanisms involve disordered keratinocyte differentiation and/or proliferation (1) In addition to the epidermal changes, some of the disorders show dermal inflammation and immunological changes The biologically active form of vitamin $D_3$, 1,25-dihydroxyvitamin $D_3$, has been demonstrated to stimulate the terminal differentiation of epidermal keratinocytes and to inhibit the proliferation of epidermal keratinocytes (3) At least in vitro 1,25-dihydroxyvitamin $D_3$ modifies differentiation by stimulating the switch of suprabasal cells into cornified cells (4) Apparently 1,25-dihydroxyvitamin $D_3$induces terminal differentiation of epidermal keratinocytes without changing their keratin gene expression in vitro (5) By modifying the epidermal growth pattern vitamin $D_3$ may improve disorders of keratinization However, X-linked ichthyosis and ichthyosis vulgaris have not responded to treatment with neither oral 1 alpha-OH-$D_3$ (6) nor topical $1,25(OH)_2D_3$ (7)
Calcipotriol is a synthetic vitamin D analogue, which is as effective as 1,25-dihydroxyvitamin $D_3$ in binding to the vitamin D receptor and in stimulating epidermal differentiation (8) Compared to 1,25-dihydroxyvitamin $D_3$, calcipotriol, however, is about 100-200 times less potent in its effect on calcium metabolism in rats (8) In clinical studies calcipotriol ointment 50 µg/g has been shown to be effective and safe for the treatment of psoriasis vulgaris (9) The purpose of the present study was to determine whether topical calcipotriol is effective, tolerated and safe in disorders of keratinization

## PATIENTS AND METHODS

**Study design and patients:** The study was designed as a prospective, randomized, double-blind, right/left comparative study of calcipotriol 50 µg/g and placebo ointment (vehicle of calcipotriol ointment), both applied twice daily The ingredients of the vehicle were disodium hydrogen phosphate, polyoxyethylene-2-stearylether, prpylene glycol, tetracemine disodium, DL-alpha-tocopherol, petrolatum, liquid parafiin and water The study was conducted in 2 centers in Denmark and in 1 center in The Netherlands Patients were not treated during summertime It divided into two phases After a wash-out phase of 2 weeks during which the patient used only an emollient, the patients were given double-blind treatment with calcipotriol ointment on one side of the body and placebo ointment on the other side for 12 weeks or until clearance on one side The criterion for including patients was a clinical and histological diagnosis of one of the following dyskeratoses ichthyosis vulgaris, X-linked ichthyosis, congenital ichthyosis, Darier's disease, pityriasis rubra pilaris, hereditary palmo-plantar keratoderma and keratosis pilaris The diagnose of X-linked ichthyosis was based on an established human steroid sulphatase deficiency (10)

Patients had symmetrically located lesions and were at least 12 years of age Excluded were patients with skin infection, atopic dermatitis, systemic treatment with retinoids, corticosteroids or PUVA within the 8 weeks period prior to the wash-out phase Also

excluded were patients with hypercalcaemia, high vitamin D or calcium intake and significant renal or hepatic disease Women of childbearing potential were only included if using an adequate method of contraception All patients gave their informed consent All affected body areas, except for the face, scalp and genital region, were treated with the study medication A maximum of 120 gram was dispensed to each side of the body per week Patients with affected areas on the face and genital region used emollient for these areas All tubes were returned and weighed to determine amount of ointment used The use of other topical medication or systemic treatment for dyskeratosis was not allowed

**Patients outcomes:** Clinical assessments were done at weeks 0, 2, 4, 8 and 12 separately for right and left side of the body The extent of the dyskeratotic involvement was recorded using the following scale 0, no involvement, 1, less than 20%, 2, 20-39%, 3, 40-59%, 4, 60-79%, and 5, 80-100% for each of the following regions (arms, legs and trunk) The investigator also assessed the severity of the following signs in ichthyosis vulgaris and keratosis pilaris scales and roughness, in X-linked ichthyosis scales, roughness and hyperpigmentation, in Darier's disease scales, erythema, papules, crust and excoriation, in pityriasis rubra pilaris scales, thickness and erythema, in hereditary palmo-plantar keratoderma scales, thickness, erythema and fissuring, and in congenital ichthyosis scales roughness and erythema These signs where assessed using the following scale 0, absent, 1, slight, 2, moderate, and 3, severe Furthermore, the investigator assessed the overall response to the treatment compared to baseline using the following 6-point scale worse, no change, slight improvement, moderate improvement, marked improvement, and cleared The patients assessed the overall response to treatment using the same scale Blood samples for analysis of hematology and blood chemistry including serum total calcium, were taken before the start of treatment, after 2 weeks treatment and at the end of treatment

**Statistical analysis:** Comparison of treatment effects at end of double-blind treatment were based on intrapatient variations of the calcipotriol treated side and the placebo treated side With respect to investigator's and patient's overall assessment the number of patients with the calcipotriol treated side superior to the placebo treated side were compared with the number of patients with the placebo treated side superior to the calcipotriol treated side by binomial tests Total sign score differences from baseline to end of double-blind treatment were calculated for both treatment sides separately and compared using one-sample t-test

For adverse events the number of patients reporting adverse events on the calcipotriol treated side only was compared with the number of patients reporting adverse events on the placebo treated side only by binomial tests

All statistical tests were two-sided and a 5% significance level was used

## RESULTS

Sixty-nine patients were randomized to receive treatment (Table I) However, 1 case included in the Darier's disease group had Hailey-Hailey disease Furtermore, 1 case of lamellar ichthyosis left the study before any collection of efficacy or safety data Therefore, efficacy and safety data were only available for 67 patients The subgroup hereditary palmo-plantar keratoderma had 20 patients randomized, while the other groups consisted of 8-12 patients each The subgroup congenital ichthyosis was heterogeneous consisting of 1 case of epidermolytic hyperkeratosis (bullous ichthyosiform erythroderma of Brocq, autosomally dominantly inherited), 2 cases of lamellar ichthyosis (nonerythrodermic autosomal recessive lamellar ichthyosis), 2 cases of Sjogren-Larsson syndrome, 1 case of ichthyosis linearis circumflexa, 2 cases of congenital ichthyosiform erythroderma (erythrodermic autosomal recessive lamellar ichthyosis), and 2 cases of ichthyosis bullosa Siemens No case of pityriasis rubra polaris was randomized At baseline there was no clinically significant difference in the total sign score on the right and left side (data not shown) Fifteen of the 69 randomized patients withdrew from the double-blind treatment (Table II) Adverse events were the reason for withdrawal in 9 of the 15 cases (see below) It was remarkable that 8 of the 12 patients with Darier's disease withdrew because of lesional/perilesional irritation, worsening of the disease In accordance with the high number of withdrawals, the mean duration of double-blind treatment was only 6 1 weeks in Darier's disease compared with 9 6 to 12 3 weeks in the other subgroups

**Table I Number and demographic data of patients randomized**

| Disease | n | No of males (%) | Age (yrs) (mean, range) |
|---|---|---|---|
| Ichthyosis vulgaris | 9 | 5 (56) | 34 1 (16-52) |
| X linked ichthyosis | 8 | 8 (100) | 34 4 (16-50) |
| Congenital ichthyosis | 11 | 5 (45) | 32 4 (16-55) |
| Darier s disease | 12 | 7 (58) | 38 6 (18-79) |
| Keratoderma | 20 | 12 (60) | 29 7 (11-57) |
| Keratosis pilaris | 9 | 1 (11) | 23 4 (16-45) |
| All patients | 69 | 38 (55) | 32 0 (11-79) |

Table III shows the investigator's overall assessment of the treatment response to calcipotriol and placebo at the end of treatment Marked improvement or clearance was observed on the calcipotriol treated side in 5 of 9 cases with ichthyosis vulgaris, in 4 of 8 cases with X-linked ichthyosis and in 8 of 10 cases with congenital ichthyosis (Figure 1), but the difference between calcipotriol and placebo was only statistically significant for X-linked ichthyosis ($p=0\ 03$) and congenital ichthyosis ($p=0\ 02$) The patient's overall assessment at the end of treatment is tabulated in the same way (Table IV) According to the patients, marked improvement or clearance was observed on the calcipotriol treated

side in 4 of 9 cases of ichthyosis vulgaris, 4 of 8 cases of X-linked ichthyosis and 7 of 10 cases of congenital ichthyosis. Only in X-linked ichthyosis did the difference between calcipotriol and placebo reach statistical significance (p=0.03).


**Fig. 1. a.** NEARLI patient with the typical phenotype consisting of large brown scales covering the trunk. **b.** After treatment, the calcipotriol-treated right side has improved markedly, whereas only slight improvement has occured on the ointment base-treated left side **c.** After treatment, a significant unilateral improvement was observed in favour of the calcipotriol-treated right leg. **d.** Some improvement was also noted in the ointment base-treated left leg.

**Table II. Reason for withdrawal from double-blind treatment**

| Disease | Adverse events (n) | Unaccept response (n) | Voluntary/ default (n) | Total (n) |
|---|---|---|---|---|
| Ichthyosis vulgaris (n=9) | 0 | 0 | 0 | 0 |
| X-linked ichthyosis (n=8) | 1 | 0 | 1 | 2 |
| Congenital ichthyosis (n=11) | 0 | 0 | 1 | 1 |
| Darier's disease (n=12) | 6 | 1 | 1* | 8 |
| Keratoderma (n=20) | 1 | 0 | 1 | 2 |
| Keratosis pilaris (n=9) | 1 | 1 | 0 | 2 |
| Total (n=69) | 9 | 2 | 4 | 15 |

* This patient had Hailey-Hailey disease

**Table III. Investigator's overall assessment of treatment response at end of treatment**

| | Calcipotriol/Placebo No. ptt. | | | | | |
|---|---|---|---|---|---|---|
| | worse | no change | slight improvement | moderate improvement | marked improvement | cleared |
| Ichthyosis vulgaris (n=9) | 0/0 | 0/0 | 2/4 | 2/4 | 5/0 | 0/1 |
| X-linked ichthyosis (n=8) | 0/0 | 1/2 | 0/4 | 3/2 | 4/0 | 0/0 |
| Congenital ichthyosis (n=11) | 0/0 | 1/4 | 0/4 | 1/1 | 8/1 | 0/0 |
| Keratoderma (n=20) | 0/0 | 9/11 | 6/5 | 3/1 | 2/2 | 0/1 |
| Keratosis pilaris (n=9) | 2/1 | 1/2 | 2/3 | 2/3 | 2/0 | 0/0 |
| Darier's disease (n=12) | 8/5 | 0/4 | 2/0 | 1/2 | 0/0 | 0/0 |

**Table IV. Patient's overall assessment of treatment response at end of treatment**

| | Calcipotriol/Placebo No. ptt | | | | | |
|---|---|---|---|---|---|---|
| | worse | no change | slight improvement | moderate improvement | marked improvement | cleared |
| Ichthyosis vulgaris (n=9) | 0/0 | 0/0 | 3/5 | 2/3 | 4/0 | 0/1 |
| X-linked ichthyosis (n=8)* | 0/1 | 0/3 | 0/2 | 3/1 | 4/1 | 0/0 |
| Congenital ichthyosis (n=10) | 0/1 | 1/4 | 0/3 | 2/1 | 0/0 | |
| Keratoderma (n=20) | 0/0 | 8/10 | 8/5 | 3/2 | 1/2 | 0/1 |
| Keratosis pilaris (n=9) | 2/1 | 2/4 | 0/0 | 3/3 | 2/1 | 0/0 |
| Darier's disease (n=12) | 8/5 | 0/3 | 1/1 | 2/1 | 0/1 | 0/0 |

* For one of the patients the calcipotriol treated side was not assessed

The total sign score, defined as the sum of the severity scores for each side of the body, could as a minimum range from 0 to 6 for ichthyosis vulgaris and keratosis pilaris and as a maximum from 0-15 for Darier's disease. The decrease in the total sign score at the end

of treatment was greater on the calcipotriol treated side than on the placebo treated side in ichthyosis vulgaris, X-linked ichthyosis and congenital ichthyosis (Figure 2).

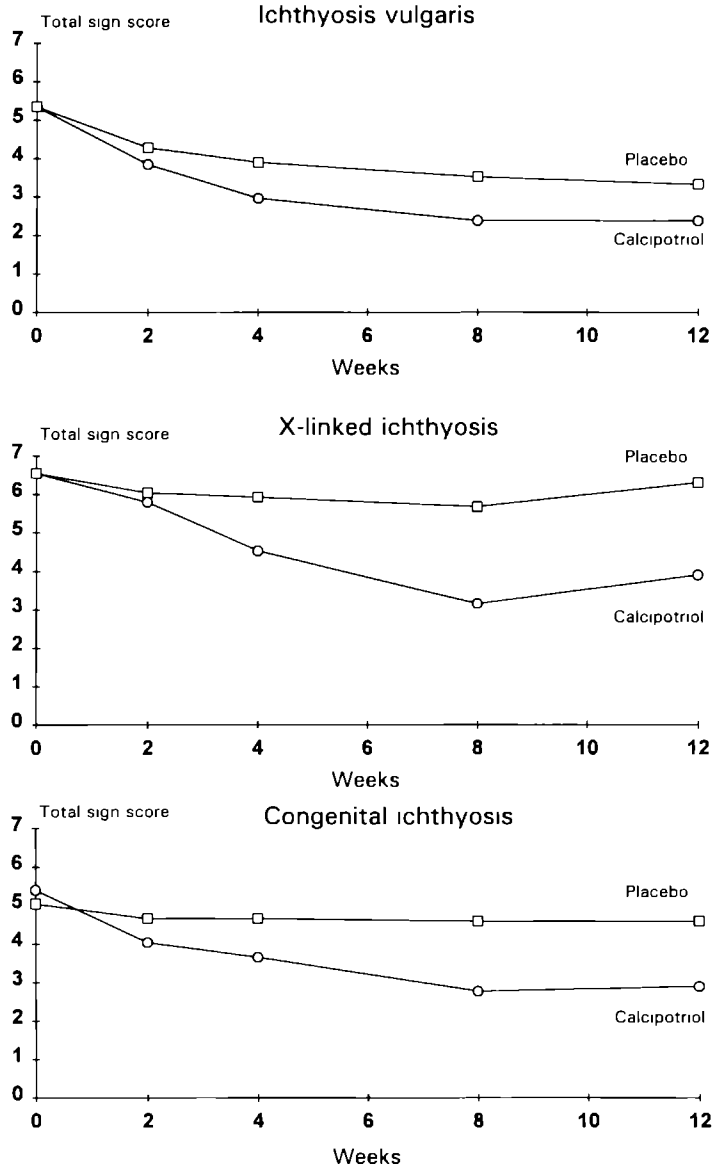


**Fig. 2.** Change in the mean total sign score during treatment with calcipotriol ointment 50 μg and placebo ointment in ichthyosis vulgaris (n=9), X-linked ichthyosis (n=8), and congenital ichthyosis (n=10)

In congenital ichthyosis this reduction in the total sign score on the calcipotriol treated side was statistically highly significant compared to the placebo treated side ($p=0.0015$) There was no statistically significant difference between calcipotriol and placebo in mean total sign score of overall assessment in other subgroups of dyskeratosis (data not shown). The adverse events reported or observed during the treatment were almost exclusively localized to the skin and consisted almost exclusively of various forms of lesional and perilesional irritation (Table V). No irritation was detected in congenital ichthyosis. Lesional/perilesional irritation was more often seen on the calcipotriol treated side ($p<0.001$). Skin irritation was most common in X-linked ichthyosis and Darier's disease. While only a single case of X-linked ichthyosis was withdrawn because of skin irritation, 6 cases of Darier's disease withdrew because of lesional/perilesional irritation. The amount of calcipotriol ointment used per week was lowest in palmo-plantar keratoderma (mean 11.8 g/week; range 2.1-25.6 g/week) and highest in congenital ichthyosis (mean 59.3 g/week; range 11.4-94.7 g/week). With respect to the change in serum total calcium from baseline to the end of treatment, the subgroup X-linked ichthyosis showed a minor, but statistically significant reduction ($p=0.045$). No subgroup showed any statistically significant increase in serum total calcium (data not shown).

**Table V. Lesional/perilesional irritation reported/observed in disorders of keratinization**

| Disease | Calcipotriol side n (%) | Pacebo side n (%) | Both sides n (%) |
|---|---|---|---|
| Ichthyosis vulgaris (9) | 2 (22) | 1 (11) | 0 |
| X-linked ichthyosis (8) | 6 (75) | 0 | 0 |
| Congenital ichthyosis (10) | 0 | 0 | 0 |
| Palmo-plantar keratoderma (20) | 5 (25) | 0 | 2 (10) |
| Keratosis pilaris (9) | 2 (22) | 0 | 2 (22) |
| Darier's disease (12) | 3 (25) | 0 | 5 (42) |
| Total number of irritations | 18 (26) | 1 (1) | 9 (13) |

## COMMENT

The results of the present study indicate that topical treatment with calcipotriol for 12 weeks is moderately efficaceous in various forms of ichthyosis. Palmo-plantar keratoderma and keratosis pilaris seem to be unresponsive to calcipotriol, whereas Darier's disease may worsen during treatment with calcipotriol, probably due to the irritating capacity of calcipotriol ointment. The presence of eroded and fissured lesions in Darier's disease may be the reason for the increased suspectability to become irritated by calcipotriol ointment. Expect for Darier's disease, the cutaneous side-effects in the dyskeratotic disorders were similar to those observed in psoriasis with respect to their nature, severity and frequency (9). Among the ichthyoses, X-linked ichthyosis and congenital ichthyosis were particularly responsive to calcipotriol. The group congenital ichthyosis is very heterogeneous, and the low number of patients in each subtype does not allow any conclusions as to any variation

in the responsiveness to calcipotriol In contrast to calcipotriol, neither oral 1 alpha-OH-$D_3$ (6) nor topical 1,25 $(OH)_2D_3$ (7) are reported to improve X-linked ichthyosis or ichthyosis vulgaris The reason for this apparent discrepancy is most likely that 1 alpha-OH-$D_3$ and 1,25-$(OH)_2D_3$ were used in too low doses Thus the applied concentration of 1,25 $(OH)_2D_3$ (1 μg/g) has no significant effect on psoriasis (12,13)

A major concern during treatment with vitamin D analogues is whether percutaneously absorbed vitamin D may change calcium metabolism In large scale studies of psoriasis, it has been found that a weekly dose of up to 100 gram calcipotriol ointment 50 μg/g does not change serum calcium levels (9) Furthermore, the biochemical markers of calcium and bone metabolism do not change in psoriatics receiving a calcipotriol dose ranging from 40 g/week (11) or 100 g/week (14) In the present study, during which patients were allowed to use up to 120 gram per week, no significant changes of serum calcium levels were found This indicates that patients with disorders of keratinization are not more likely than psoriatics to develop hypercalcaemia after topical application of calcipotriol, However, it should be born in mind that only one half of the affected skin lesions were treated with calcipotriol In some of the patients with more widespread disease, it might have been possible to treat all lesions with the allowed amount of calcipotriol (120 g/week) As it is recommended for psoriasis, calcipotriol ointment 50 μg/g was applied twice daily in the dyskeratoses It is, however, possible that application of calcipotriol ointment once daily is efficaceous for the treatment of ochthyosis If so, 120 g of calcipotriol ointment per week might be sufficient in most patients

Vitamin D analogues have multiple actions on the cellular and molecular level, and it has still not been sorted out whether vitamin D analogues work in psoriasis by modifying keratinocyte differentiation/proliferation or whether their immunosuppressive effects are important for their anti-psoriatic effect The fact that treatment with calcipotriol can improve the ichthyoses supports the idea that calcipotriol in vivo has an effect on epidermal differentiation and/or proliferation independent of immunomodulation

It can be concluded that topical treatment with calcipotriol 50 μg/g for 12 weeks can induces a moderate improvement of various forms of ichthyoses While calcipotriol is well-tolerated in ichthyosis, it may worsen Darier's disease, probably due to its irritating capacity Although ichthyosis may be reponsive to calcipotriol treatment, large-scale studies, but especially long-term studies, are needed to confirm the present results If calcipotriol ointment is used for the treatment of ichthyosis, it is important that the weekly dose is kept below 120 gram If calcipotriol is applied twice daily, about 15-20% of the body surface can be treated, but application once daily makes it possible to increase the treated skin area The safety and efficacy of this alternative approach warrants further investigation If topical calcipotriol is found to be efficaceous and safe in long-term large-scale studies, this treatment may be of great benefit to patients severely affected by ichthyosis Also, it should be investigated whether it is beneficial to combine calcipotriol with a retinoid In addition to a simple additive effect of these two classes of compounds, an interaction might occur on the molecular level leading to a synergistic effect (15)

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# Summary, Discussion and Conclusion

At the end of the general introduction three questions were addressed with respect to the nosology and treatment of monogenic disorders of keratinization Chapters 2, 3 and 4 provide the original contributions regarding these questions Here, these contributions will be discussed In the appendix guidelines for classification of monogenic disorders of keratinization will be presented

**Question 1:**
**To what extent do our clinical descriptions of phenotypes, regarding disorders of keratinization, refine and or simplify the existing nosology of monogenic disorders of keratinization?**

The case reports presented in Part I of this study demonstrate that the list of disorders of keratinization is prone to further extension On the other hand, unifying concepts have been developed based on clinical descriptions The cases described in Part I call for a critical reappraisal of some of the existing concepts In the case description of ichthyosis bullosa of Siemens (Part I, chapter 1) a precise clinical, histopathological, and ultrastructural analysis clearly demonstrates that in morphological terms this condition is distinct from bullous ichthyosiform erythroderma of Brocq Ironically, this paper was rejected by the British Journal of Dermatology, as the referee was of the opinion that splitting within the bullous ichthyoses group was not relevant However, subsequent molecular genetic analyses (Part II, chapters 4 and 5) revealed that ichthyosis bullosa of Siemens is an entity distinct from bullous congenital ichthyosiform erythroderma of Brocq From a therapeutic point of view this distinction is relevant as the two conditions show a different response to retinoids (Part II, chapter 2)
On the other hand the molecular genetic analysis in Part II, chapters 4 and 5 demonstrated that the so-called autosomal dominant ichthyosis exfoliativa is not an entity but is identical to ichthyosis bullosa of Siemens
The cases described in chapters 4, 5 and 6 of Part I further substantiate the concept of cutaneous mosaicism It is already known that an individual with the epidermolytic type of epidermal naevus[1], is at risk for having a germ-line mosaicism and may transmit the underlying gene to the next generation in the form of a bullous congenital ichthyosiform erythroderma of Brocq In this disease an autosomal dominant mutation is involved The naevus comiculatus (Part I, chapter 4) and the porokeratotic eccrine ostial and dermal duct naevus (Part I, chapter 6) are probably also caused by somatic mutations Recently a child with an erythrodermic type of ichthyosis was demonstrated whose mother had an extensive eccrine ostial and dermal duct naevus [2] The transmission of CHILD-syndrome from mother to daughter in chapter 5 of Part I provides further support to the concept of X-linked dominant inheritance with lethality for male embryos Chapter 6 of Part I demonstrates that coexistence of two genetic diseases does not necessarily imply a genetic link, but may be due to isomorphic elicitation

**Question 2:**

**To what extent do our immunohistochemical and molecular findings refine and or simplify the existing nosology of monogenic disorders of keratinization?**

Part II provides new immunohistochemical and molecular data on aspects of epidermal growth, keratinization, and inflammation in various disorders of keratinization. Disorders of keratinization characterized clinically and histopathologically by inflammation (Table I) showed an extended expression of keratin 5 and 14, an induction of keratin 6 and 16, and in some cases of keratin 17 (Part II, chapter 1) These disorders demonstrated an increased expression of the extracellular matrix component tenascin as well (Part II, chapters 2 and 3). It is tempting to hypothesize that both keratin and tenascin expresssion in disorders of keratinization are modulated by concommittant inflammation. The interrelation between inflammation and abnormal keratinization has been discussed in chapter 1 of Part II Keratin 6, 16, and 17 have been shown to be proliferation associated keratins. As the inflammatory disorders of keratinization as a rule are hyperproliferative, the changes of the keratin pattern can be interpreted as a reflection of a hyperproliferative epidermis. As such, it is most likely that these findings do not form the primary genetic defect, but are probably epiphenomens.

**Table I. Disorders of keratinization with a normal and abnormal profile of keratins 5, 14, 6, 16, 17 and tenascin.**

| Disorders with abnormal expression of keratins and tenascin | Disorders with normal expression of keratins and tenascin |
|---|---|
| • Erythrodermic lamellar ichthyosis<br>• Bullous congenital ichthyosiform erythroderma<br>• Ichthyosis bullosa of Siemens<br>• Restrictive dermopathy<br>• CHILD-syndrome<br>• Collodion baby<br>• Harlequin ichthyosis | • Autosomal dominant ichthyosis vulgaris<br>• X-linked recessive ichthyosis<br>• Nonerythrodermic lamellar ichthyosis |

Molecular biological techniques are capable of elucidating the primary defect. Recently, in patients with bullous congenital ichthyosiform erythroderma of Brocq mutations were found in either the gene coding for keratin 1 or keratin 10. The epidermolytic type of palmoplantar keratoderma, type Vörner, appeared to be caused by a mutation in keratin 9, a keratin present in the epidermis of palms and soles. Despite of its clinical and histological characteristics ichthyosis bullosa of Siemens was not commonly accepted as an entity. Mutation analysis however, demonstrated that the disease is due to mutations in a unique keratin, namely keratin 2e (Part I, chapter 5). Further studies on the genetics of monogenic disorders of keratinization, including gene mapping and mutation analysis, are the approaches which will provide the rationale for the nosology of genetically determined disorders of keratinization.

A precise clinical diagnosis of a disorder of keratinization is not art for art's sake, but is necessary for genetic counseling,for estimating the prognosis, and for selecting therapy

**Question 3:**

**To what extent are systemic acitretin, topical 13-cis retinoic acid, and topical vitamin $D_3$ (all ligands for members of the steroid receptor superfamily) effective in the treatment of disorders of keratinization?**

In Part III (chapters 1-6) the therapeutic potential of two ligands for the steroid receptor superfamily, retinoids and vitamin $D_3$, is described with respect to the treatment of monogenic disorders of keratinization Table II summarizes the therapeutic response of the various disorders of keratinization to systemic treatment with acitretin and the dosage which proved to be advisable Our findings demonstrated that several disorders of keratinization are a good indication for systemic retinoid therapy Dose management however, depends on the diagnosis The studies in Part III showed that ichthyosis bullosa of Siemens requires a lower dose of systemic retinoids than bullous congenital ichthyosiform erythroderma The same is true for erythrodermic lamellar ichthyosis compared to non-erythrodermic lamellar ichthyosis It has been reported that a large interindividual variation exists in the treatment of M Darier Therefore, it is advisable to initiate treatment in this condition with a low dose schedule

**Table II Response of various disorders of keratinization to treatment with acitretin**

| Diagnosis | Response | Optimal Dosage Range (mg/day) |
|---|---|---|
| Ichthyosis bullosa of Siemens* | excellent | 10-25 mg |
| Bullous congenital ichthyosiform erythroderma* | excellent | 40-60 mg* |
| Nonerythrodermic lamellar ichthyosis* | mild excellent | 10-55 mg |
| Erythrodermic lamellar ichthyosis* | excellent | 10-25 mg |
| Erythrokeratodermia variabilis* | excellent | 25-35 mg |
| Mal de Maleda@ | moderate | 10-30 mg |
| M Greither§ | excellent | 25-35 mg |
| M Darier# | moderate-excellent | 10-60 mg |

* etretinate (the ethyl-ester of acitretin)
+ present thesis Part III, chapters 1-3
@ Br J Dermatol 1992,127 191-192
§Eur J Dermatol 1992,2 503-505
# Br J Dermatol 1989,121 375 379

The profile of systemic side-effects was not disease associated However, "irritation" and aggrevation of the inflammatory component occur in Darier's disease and erythrodermic lamellar ichthyosis if the dosage of acitretin is too high

Since cytostatics that have an effect on proliferation do not have a clinical response in disorders of keratinization, the beneficial effect of retinoids is probably due to a direct effect on the differentiation process and not on epidermal proliferation Topical retinoids

like 13-cis-retinoic may be useful in some cases (Part III, chapters 4 and 5). In particular the efficacy in Darier's disease and in some patients with lamellar ichthyosis is impressive. The limitation of topical all-trans-retinoic acid is its irritative potential. As has been shown in chapters 4 and 5 of Part III, 13-cis-retinoic acid has a more favourable efficacy/irritancy ratio. New compounds such as the arotinoids have an even lower irritative potential and therefore might be promising candidates as topical treatment of disorders of keratinization for the future.

The vitamin $D_3$ analogue calcipotriol is a well established antipsoriatic drug. In chapter 6 of Part III calcipotriol proved to be effective in various disorders of keratinization (Table III). The study with topical vitamin $D_3$ showed that this treatment can be used with success in the treatment of congenital ichthyoses and to a lesser extent in X-linked recessive ichthyosis.

**Table III. Response of various disorders of keratinization to calcipotriol ointment**

| | Beneficial | No effect | Deterioration |
|---|---|---|---|
| Ichthyosis vulgaris | ± | | |
| X-linked ichthyosis | + | | |
| Darier White disease | | | ++ |
| Keratoderma | | + | |
| Keratosis pilaris | | + | |
| Congenital ichthyosis | ++ | | |

It is striking that all the investigated drugs that appear to have a beneficial effect in monogenic disorders of keratinization belong to the ligands of the steroid receptor super family. In this respect it would be interesting to study the therapeutical effect of other ligands of the members of the steroid receptor super family like topical oestrogens, androgens, and thyroxin. An important breakthrough in the treatment of disorders of keratinization has been provided by the cytochrome P450 inhibitor Liarozole® (Janssen Pharmaceutica, Beerse, Belgium). This inhibitor increases the endogenous levels of vitamin A in the tissues. A recent study, carried out by the genodermatology research group of Nijmegen, revealed that clearing occurred in all 12 patients with various forms of ichthyosis during treatment with this compound.[3]

## CONCLUSION

The future nosology of monogenic disorders of keratinization has to be defined based on the gene defects involved. Descriptive studies using markers for epidermal differentiation and proliferation so far have not revealed a valuable adjunct to clinical description and genetic definition. Future studies however, might indicate new candidate genes. New treatments interfering with the steroid-receptor-superfamily are likely to broaden the spectrum of treatments for disorders of keratinization.

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3 Lucker GPH, Heremans A, Boegheim PJ, Steijlen PM Oral liarozole treatment of hereditary ichthyoses an open labelled clinical and immunohistochemical study 1994, Submitted

# Samenvatting, Discussie en Conclusie

In de algemene inleiding van dit proefschrift zijn drie vragen gesteld, die betrekking hebben op de nosologie en de behandeling van keratinisatiestoornissen. In deel I, II en III zijn de publicaties ondergebracht welke betrekking hebben op deze vraagstellingen In dit hoofdstuk worden deze studies bediscussieerd in het kader van de geformuleerde vraagstelling In de appendix wordt een handleiding gepresenteerd, die kan helpen bij de classificatie van de monogene keratinisatiestoornissen

**Vraagstelling 1:**
**In welke mate verfijnen of simplificeren onze klinische beschrijvingen van keratinisatiestoornissen de bestaande nosologie van de monogene keratinisatie-stoornissen?**

De beschrijvingen van de patienten in deel I laten zien dat de lijst van verhoorningsstoornissen zich steeds verder uitbreidt Op grond van klinische beschrijvingen zijn algemene concepten ontwikkeld De patienten, beschreven in deel I, helpen bij een kritische herwaardering van enkele bestaande concepten In de beschrijving van patienten met ichthyosis bullosa Siemens (deel I, hoofdstuk 2) laat een precíese klinische, histologische en ultrastructurele analyse zien, dat deze aandoening op morphologische gronden moet worden onderscheiden van de bulleuze congenitale ichthyosiforme erythrodermie van Brocq Dit artikel werd door de British Journal of Dermatology geweigerd, omdat de referee van mening was dat een onderscheid binnen de groep van bulleuze ichthyoses niet relevant was Ook in de Amerikaanse literatuur wordt het onderscheid niet gemaakt en worden beide aandoeningen geschaard onder het begrip epidermolytische hyperkeratose, dit is echter een histologische bevinding Moleculair biologisch onderzoek heeft echter aangetoond dat ichthyosis bullosa Siemens een entiteit is die verschilt van de bulleuze congenitale ichthyosiforme erythrodermie van Brocq (deel II, hoofdstuk 4 en 5) Wat betreft de behandeling is dit verschil ook relevant, omdat de twee aandoeningen een verschillende respons laten zien op retinoiden (deel III hoofdstuk 2)
Aan de andere kant toonde het moleculair biologisch onderzoek in hoofdstuk 4 en 5 van deel II aan dat de zogenaamde autosomaal dominante ichthyosis exfoliativa geen entiteit is, maar identiek is aan ichthyosis bullosa Siemens
De beschrijvingen van patienten in hoofdstuk 4, 5 en 6 van deel I geven een verdere onderbouwing van het concept van het cutane mozaiek Het is bekend dat een ouder met het epidermolytische type van epidermale naevus, het risico heeft op een gonadaal mozaiek[1] Dit betekent dat de ouder het risico heeft op het krijgen van een kind met bulleuze congenitale ichthyosiforme erythrodermie van Brocq De naevus berust op een somatische mutatie, vroeg in de embryogenese De naevus comiculatus (deel I, hoofdstuk 4) is waarschijnlijk ook het gevolg van een somatische mutatie vroeg in de embryogenese De "porokeratotic eccrine ostial and dermal duct" naevus (de ı I, hoofdstuk 6) wordt

waarschijnlijk ook veroorzaakt door een somatische mutatie. Recent werd een kind gedemonstreerd met een erythrodermische vorm van ichthyosis, waarvan de moeder een "porokeratotic eccrine ostial and dermal duct" naevus had[2]. Het kind overleed aan de aandoening op zeer jonge leeftijd. De overerving van het CHILD syndroom van moeder op dochter levert verder ondersteuning aan het concept dat het bij dit syndroom gaat om een X-gebonden dominante overerving, die letaal is voor mannelijke embryos (deel I, hoofdstuk 5). Hoofdstuk 6 van deel I laat zien dat het naast elkaar bestaan van twee genetische aandoeningen niet noodzakelijkerwijs een genetische "link" inhoudt, maar dat in dit geval de psoriasis ook het gevolg kan zijn van het isomorph prikkelphenomeen.

**Vraagstelling 2:**
**In welke mate verfijnen of simplificeren onze immunologische en moleculaire bevindingen de bestaande nosologie van de monogene keratinisatiestoornissen?**

Deel II levert nieuwe immunohistochemische en moleculaire gegevens over epidermale groei, keratinisatie en ontsteking bij de verschillende keratinisatiestoornissen. Keratinisatiestoornissen die klinisch en histopathologisch gekenmerkt worden door ontsteking (Tabel I) laten een uitgebreidere expressie zien van keratine 5 en 14, een inductie van keratine 6 en 16 en in sommige gevallen van keratine 17 (deel II, hoofdstuk 1). Deze aandoeningen laten ook een verhoogde expressie zien van tenascine (deel II, hoofdstuk 2 en 3) Het is verleidelijk om te hypothetiseren dat zowel de keratine expressie als de tenascine expressie gemoduleerd wordt door de aanwezigheid van ontsteking. Het verband tussen ontsteking en de abnormale keratine expressie is bediscussieerd in hoofdstuk 1 van deel II. Keratine 6, 16 en 17 zijn proliferatie-geassocieerde keratines. Aangezien keratinisatiestoornissen met een ontstekingscomponent in de regel hyperproliferatief zijn, kan de verandering in het keratinepatroon worden beschouwd als een weerspiegeling van een hyperproliferatieve epidermis. Het is dan ook zeer waarschijnlijk dat deze bevindingen niet het primaire defect vormen, maar epiphenomenen zijn.

**Tabel I. Keratinisatiestoornissen met een normaal en abnormaal expressiepatroon van keratine 5, 14, 6, 16, 17 en tenascine.**

| Aandoeningen met abnormale expressie van keratines en tenascine | Aandoeningen met normale expressie van keratines and tenascine |
|---|---|
| • Erythrodermische lamellaire ichthyosis<br>• Bulleuze congenitale ichthyosiforme erythrodermie<br>• Ichthyosis bullosa van Siemens<br>• "Restrictive dermopathy"<br>• CHILD-syndroom<br>• "Collodion baby"<br>• Harlekein ichthyosis | • Autosomaal dominante ichthyosis vulgaris<br>• X-gebonden recessieve ichthyosis<br>• niet-erythrodermische lamellaire ichthyosis |

Moleculair-biologische technieken zijn in staat het primaire defect op te helderen. Recent werden bij patienten met een bulleuze congenitale ichthyosiforme erythrodermie van Brocq, mutaties gevonden in hetzij het gen coderend voor keratine 1 , hetzij het gen coderend voor keratine 10. De epidermolytische vorm van palmoplantaire hyperkeratose, type Vörner, blijkt te worden veroorzaakt door een mutatie in het keratine 9, een keratine aanwezig in de epidermis van handpalmen en voetzolen. Ondanks de kenmerkende klinische en histologische eigenschappen, werd ichthyosis bullosa van Siemens niet beschouwd als een entiteit. Mutatieanalyse toonde echter aan dat de ziekte het gevolg is van mutaties in een uniek keratine, namelijk keratine 2e (deel II, hoofdstuk 5). Verder onderzoek naar de genetica van keratinisatiestoornissen waaronder genkartering en mutatieanalyse zal de basis verschaffen voor de nosologie van genetisch bepaalde keratinisatiestoornissen.
Een juiste klinische diagnose van een keratinisatiestoornis in niet een kwestie van "l' art pour l' art", maar is nodig om de prognose te bepalen, voor het geven van genetisch advies, en voor het selecteren van de behandeling.

**Vraagstelling 3:**
**In welke mate is toepassing van systemisch acitretin, topisch 13-cis-retinoic acid, en topisch vitamine D3 (allen liganden van de "steroid receptor superfamily") effectief bij de behandeling van keratinisatiestoornissen?**

In deel III (hoofdstuk 1-6) wordt het therapeutisch potentieel beschreven van twee liganden van de "steroid receptor superfamily" bij de behandeling van monogene keratinisatiestoornissen: retinoiden en vitamine $D^3$. Tabel II vat de therapeutische respons samen van de verschillende keratinisatiestoornissen op systemische behandeling met acitretin en de te adviseren dosis. Onze bevindingen tonen aan dat meerdere keratinisatiestoornissen een goede indicatie vormen voor behandeling met systemische retinoiden. De dosis is echter afhankelijk van de diagnose. De studies van deel III laten zien dat voor de behandeling van ichthyosis bullosa Siemens een lagere dosis noodzakelijk is dan voor de bulleuze congenitale ichthyosiforme erythrodermie van Brocq. Hetzelfde geldt voor de erythrodermische lamellaire ichthyosis vergeleken met de niet-erythrodermische lamellaire ichthyosis. Er is gepubliceerd dat er een grote interindividuele variatie bestaat bij de behandeling van de ziekte van Darier. Daarom wordt er bij deze aandoening geadviseerd met een lage dosis te starten.
Het profiel van met name de mucocutane bijwerkingen is niet afhankelijk van de ziekte, maar van de dosering. Bij de erythrodermische vorm van lamellaire ichthyosis en de ziekte van Darier, die een lagere dosis vragen, worden naast mucocutane bijwerkingen, irritatie en een verergering van de ontstekingscomponent waargenomen, wanneer een te hoge dosis wordt gegeven.
Het gunstige effect van retinoiden is waarschijnlijk het gevolg van een direct effect op het differentiatieproces en niet van een werking op de epidermale proliferatie, omdat

cytostatica, die een effect hebben op proliferatie, bij keratinisatiestoornissen geen verbetering geven.

**Tabel II. De respons van de verschillende keratinisatiestoornissen op acitretine**

| Diagnose | Respons | Optimale Dosis "range" (mg/dag) |
|---|---|---|
| Ichthyosis bullosa van Siemens* | zeer goed | 10-25 mg |
| Bulleuze congenitale ichthyosiforme erythrodermie+ | zeer goed | 40-60 mg* |
| Niet-erythrodermische lamellaire ichthyosis+ | matig-zeer goed | 10-55 mg |
| Erythrodermische lamellaire ichthyosis+ | zeer goed | 10-25 mg |
| Erythrokeratodermia variabilis+ | zeer goed | 25-35 mg |
| Mal de Maleda@ | redelijk | 10-30 mg |
| M Greither$ | zeer goed | 25-35 mg |
| M Darier# | redelijk-zeer goed | 10-60 mg |

* etretinate (de ethyl-ester van acitretine)
+ dit proefschrift deel III, hoofdstuk 1-3
@ Br J Dermatol 1992,127:191-192
$ Eur J Dermatol 1992;2:503-505
# Br J Dermatol 1989,121.375-379

Locale retinoiden zoals "13-cis-retinoic acid" kunnen in sommige gevallen van nut zijn (deel III, hoofdstuk 4 en 5). Met name de effectiviteit bij de ziekte van Darier en bij sommige patienten met lamellaire ichthyosis was indrukwekkend. Een beperking van locaal all-trans-retinoic acid was tot dusver de irritatieve bijwerking. Zoals werd aangetoond in de hoofstuk 4 en 5 van deel III heeft "13-cis-retinoic acid" een gunstiger therapeutische (effect/irritatie) breedte. Nieuwe stoffen zoals de arotinoiden hebben een nog minder irritatieve werking en zijn mogelijk veelbelovende kandidaten voor de topische behandeling van keratinisatiestoornissen in de toekomst.
Locale applicatie van het vitamine $D^3$ analoog calcipotriol is een gevestigde behandeling voor psoriasis. In hoofdstuk 6 van deel III bleek calcipotriol effectief bij de behandeling van verscheidene keratinisatiestoornissen (Tabel III). De studie met topisch vitamine D3 liet zien dat deze behandeling met succes kan worden toegepast bij de behandeling van congenitale ichthyosis, en in mindere mate bij de X-gebonden recessieve ichthyosis.

**Tabel III. De respons van de verschillende keratinisatiestoornissen op calcipotriol zalf**

| | Gunstig | Geen effect | Verergering |
|---|---|---|---|
| Ichthyosis vulgaris | ± | | |
| X-gebonden ichthyosis | + | | |
| de ziekte van Darier | | | ++ |
| Keratoderma | | + | |
| Keratosis pilaris | | + | |
| Congenitale ichthyosis | ++ | | |

Het is interessant dat alle bestudeerde geneesmiddelen, die een gunstig effect hebben bij de behandeling van keratinisatiestoornissen behoren tot de liganden voor de leden van de "steroid receptor superfamily". In dit verband is het aangewezen om het therapeutisch

effect van andere liganden voor de leden van de "steroid receptor superfamily", zoals topische oestrogenen, androgenen en thyroxine te bestuderen. Een belangrijke doorbraak bij de behandeling van keratinisatiestoornissen is geleverd door de cytochroom P450 inhibitor Liarozole® (Janssen Pharmaceutica, Beerse, Belgie). Deze inhibitor verhoogt de endogene vitamine A spiegel in de weefsels. Een door ons recent uitgevoerde studie toonde aan dat dit middel bij alle 12 deelnemende patienten met een ernstige vorm van ichthyosis, een zeer goede verbetering gaf[3].

## CONCLUSIE

De nosologie van de monogene keratinisatiestoornissen zal in de toekomst vooral gebaseerd zijn op de oorzakelijke gendefecten. Het toepassen van merkstoffen voor epidermale differentiatie en proliferatie heeft tot dusver slechts een geringe bijdrage geleverd tot een betere definitie van de verschillende keratinisatiestoornissen Dergelijk descriptief onderzoek kan echter behulpzaam zijn bij het vaststellen van kandidaatgenen.
Nieuwe behandelingen, die interfereren met de leden van de "steroid receptor superfamily", zullen mogelijk het therapeutisch arsenaal voor de behandeling van keratinisatiestoornissen uitbreiden.

## REFERENCES


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3 Lucker GPH, Heremans A, Boegheim PJ, Steijlen PM Oral liarozole treatment of hereditary ichthyoses an open labelled clinical and immunohistochemical study 1994, Submitted

# Appendix

The monogenic disorders of keratinization display an enormous genetic heterogeneity and a phenotypic variation even in the same genetic type. A correct clinical diagnosis is not a mere academic excercise but is necessary for adequate genetic counseling, for estimating the prognosis and selecting the right therapy. Missing a diagnosis for instance in Refsum's disease may have dramatic consequences for the patient, since a special diet can prevent neurological damage.

An integrated approach is necessary for giving a correct diagnosis. Therefore, the appendix contains tables with clinical presentations, and histopathological (lightmicroscopical and electronmicroscopical), biochemical, and genetic findings. They have been simplified in order to be didactic. Further details can be found in the references. The tables are based on those published by Traupe on ichthyosis. Here other disorders of keratinization are included.

Obviously this classification is provisional and must be modified continually as new information becomes available. Over the years to come, new types of disorders of keratinization will emerge or disappear.

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**Table I Clinical presentations of monogenic disorders of keratinization**

| | inheritance | no associated features | associated features |
|---|---|---|---|
| ichthyosis, vulgar | AD | • autosomal dominant ichthyosis vulgaris | |
| | AR | | • Refsum's disease<br>• multiple sulfatase deficiency |
| | X-linked | • X-linked recessive ichthyosis | • associated steroid sulfatase deficiency |
| ichthyosis, congenital | AD | • autosomal dominant lamellar ichthyosis<br>• bullous ichthyosiform erytroderma of Brocq<br>• ichthyosis bullosa of Siemens<br>• ichthyosis hystrix of Curth Macklin | • KID syndrome |
| | AR | • erythrodermic lamellar ichthyosis<br>• non-erythrodermic lamellar ichthyosis | • neutral lipid storage disease<br>• Sjogren-Larsson syndrome<br>• Tay syndrome |
| | AR/AD? | • harlequin fetus | |
| | X-linked | | • X-linked dominant ichthyosis |
| palmoplantar hyperkeratosis, diffuse | AD | • Unna-Thost<br>• Greither<br>• Vorner<br>• Sybert | • Vohwinkel<br>• Howel-Evans syndrome<br>• Huriez syndrome<br>• Clouston syndrome<br>• PPK and sensorineural deafness<br>• Olmsted syndrome |
| | AR | • Mal de Meleda<br>• Gamborg Nielsen<br>• Nagashima<br>• acral keratoderma | • Papillon-Lefevre syndrome<br>• Bureau-Barriere-Thomas<br>• congenital atrichia, palmoplantar hyperkeratosis mental retardation, and early loss of teeth |
| palmoplantar hyperkeratosis, nummular/linear | AD | • Wachters<br>• PPK nummularis | • Richner-Hanhart<br>• pachyonychia congenita<br>• focal plamoplantar and oral mucosa hyperkeratosis syndrome |
| | AR | | • pachyonychia congenita<br>• Jakac-Wolf |
| | ? | | • acantholytic ectodermal dysplasia |
| palmoplantar hyperkeratosis papular | AD | • Davies-Colley<br>• acrokaratoelastoidosis<br>• focal acral hyperkeratosis | • Hanhart |
| | AR | | • Schopf-Schulz-Passarge |

**Table I (cont.). Clinical presentations of monogenic disorders of keratinization**

| inheritance | no associated features |
|---|---|
| AD | • erythrokeratodermia variabilis<br>• erythrokeratodermia symmetrica et progressiva |
| | • porokeratosis of Mibelli<br>• porokeratosis palmaris, plantaris et disseminata<br>• disseminated superficial actinic porokeratosis |
| | • Darier's disease<br>• keratitis, ichthyosis-like hyperkeratosis, and deafness syndrome |
| | • keratosis pilaris* |
| AR | • familial continual skin peeling syndrome |
| XR | • keratosis pilaris decalvans = keratosis follicularis spinulosa decalvans (Siemens)** |

* can occur isolated and in some syndromes and conditions
** can occur isolated or associated with photophobia

**Table II. Mozaic patterns in the monogenic disorders of keratinization**

| inheritance | disorder of keratinization |
|---|---|
| AD | • epidermolytic epidermal naevus<br>• acantholytic epidermal naevus<br>• linear porokeratosis |
| X-linked dominant lethal for affected male embryos | • CHILD-syndrome<br>• X-linked dominant ichthyosis |
| unknown* | • porokeratotic eccrine ostial epidermal and dermal duct naevus<br>• naevus comiculatus |

* monogenic basis unknown

**Table III. Prominent lightmicroscopical features**

| feature | diagnosis |
|---|---|
| reduced or absent granular layer | • autosomal dominant ichthyosis vulgaris<br>• Comel-Netherton syndrome<br>• Tay syndrome<br>• X-linked dominant ichthyosis |
| epidermolytic hyperkeratosis | • bullous congenital ichthyosiform erythroderma of Brocq<br>• ichthyosis bullosa of Siemens<br>• ichthyosis hystrix of Curth-Macklin<br>• palmoplantar hyperkeratosis of Vorner<br>• keratosis palmoplantaris nummularis |
| lipid droplets in basal and suprabasal keratinocytes (Sudan red) | • Refsum's disease |
| vacuolated granulocytes | • multiple sulfatase deficiency syndrome<br>• neutral lipid storage disease |
| acantholysis | • Darier's disease<br>• naevus comiculatus<br>• acantholytic ectodermal dysplasia |
| cornoid lamella | • porokeratosis |
| splitting between granular layer and stratum corneum | • continuous skin peeling syndrome |
| banding pattern of hair in polarizing light | • Tay syndrome |
| trichorrhexis invaginata | • Comel-Netherton syndrome |

**Table IV. Electronmicroscopical features**

| features | diagnosis |
|---|---|
| absent or abortive keratohyalin granules | • autosomal dominant ichthyosis vulgaris |
| clumping of tonofilaments | • bullous congenital ichthyosiform erythroderma of Brocq<br>• ichthyosis bullosa of Siemens<br>• ichthyosis hystrix of Curth-Macklin<br>• keratosis palmoplantaris type Vorner<br>• keratosis palmoplantaris nummularis |
| lipid vacuoles in the stratum corneum | • erythrodermic lamellar ichthyosis (type A) |
| cholesterol crystals in corneocytes | • erythrodermic lamellar ichthyosis (type B) |
| laminated membrane structures, vesicular lamellar bodies | • non erythrodermic lamellar ichthyosis (type A) |
| laminated structures different from the type NELI-A | • non erythrodermic lamellar ichthyosis (type B) |
| increased transition layer | • autosomal dominant lamellar ichthyosis |

**Table V. Biochemical features**

| features | diagnosis |
|---|---|
| arylsulfatase C deficiency<br>steroid sulfatase deficiency | • X-linked recessive ichthyosis |
| increased creatine kinase | • neutral lipid storage disease |
| reduced fatty alcohol $NAD^+$ oxidoreductase activity | • Sjogren-Larsson syndrome |
| reduction or absence of filaggrin and profilaggrin | • autosomal dominant ichthyosis vulgaris |
| phytanic acid oxidase deficiency | • Refsum's disease |
| tyrosine aminotransferase deficiency | • Richner-Hanhart syndrome |

**Table VI. Location of gene defects in disorders of keratinization**

| | |
|---|---|
| chromosome 12, 12q11-13,<br>mutation in keratin 1<br>chromosome 17, 17q12-21<br>mutation in keratin 10 | • bullous congenital ichthyosiform erythroderma of Brocq |
| chromosome 12, 12q11-13<br>mutation in keratin 2e | • ichthyosis bullosa of Siemens |
| chromosome 17, 17q12-21<br>mutation in keratin 9 | • keratosis palmoplantaris Vorner |
| X-chromosome, Xp22 3<br>X-chromosome<br>X-chromosome | • X-linked recessive ichthyosis<br>• X-linked dominant ichthyosis<br>• CHILD-syndrome |

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# Curriculum Vitae

De schrijver van dit proefschrift werd op 8 januari 1958 te Den Haag geboren. Hij volgde de lagere school te Parijs, Algiers, Port-Gentil en Den Haag. In 1976 behaalde hij het einddiploma Gymnasium ß aan het Sint Jans College te Den Haag, waarop hij in 1976 begon met de studie geneeskunde aan de Rijksuniversiteit Leiden. Het artsexamen werd afgelegd in augustus 1983.

Van oktober 1983 tot januari 1985 werkte hij op het Pathologisch Laboratorium van de Rijksuniversiteit Leiden en was lid van de werkgroep "Naevi en Melanomen" onder leiding van prof.dr. D.J. Ruiter. Tijdens deze periode ontving hij een beurs van de stichting "de Drie Lichten". Van januari 1985 tot 1 september 1986 was hij in opleiding tot dermatoloog op de afdeling Dermatologie van het Academisch Ziekenhuis Groningen (opleider prof.dr. J.P. Nater). Van 1 september 1986 tot 1 juni 1987 was hij klinisch en wetenschappelijk werkzaam op de afdeling Dermatologie van het Academisch Ziekenhuis Nijmegen onder leiding van prof.dr. R. Happle, tevens volgde hij een stage immuno-dermatologie aan de Universitäts Hautklinik te Münster onder leiding van prof.Dr. E-B Bröcker. Gebieden van onderzoek waren congenitale naevi en alopecia areata. In juni 1987 werd de opleiding tot dermatoloog hervat (opleider prof.dr. R. Happle). Na zijn inschrijving in het specialistenregister op 1 juli 1989 werd hij op de afdeling Dermatologie van het Academisch Ziekenhuis Nijmegen aangesteld als chef de clinique. Naast de dermatologie in de volle breedte is zijn interessegebied de genodermatologie. Hij geeft leiding aan het spreekuur genodermatologie en aan het onderzoek op het gebied van de keratinisatiestoornissen.

Hij is getrouwd met Jeanne-Marie Smits en is de trotse vader van de één-jarige Annemarijn.

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# STELLINGEN

## behorende bij het proefschrift
## "The Monogenic Disorders of Keratinization"

1 Epidermolytische hyperkeratose is een histologische bevinding en niet een diagnose.

2. Ichthyosis bullosa van Siemens is een vorm van ichthyosis die moet worden onderscheiden van de bulleuze congenitale ichthyosiforme erythrodermie van Brocq

3. Het beschrijvend dermatologisch onderzoek van de cutane mozaieken leidt tot nieuwe genetische concepten.

4. Beschrijvend onderzoek met behulp van immunohistochemische merkstoffen leidt slechts zelden tot het aanwijzen van kandidaatgenen.

5. De monogene keratinisatiestoornissen kunnen het best worden geclassificeerd op basis van hun primaire gendefecten.

6 Liganden voor de "steroid receptor superfamily" zijn effectief bij de behandeling van monogene keratinisatiestoornissen.

7. Bij dysplastische (atypische) naevi zijn meerdere genen betrokken.

8. Het minst dysplastische van de promovendus zijn zijn naevi.

9. Topische immuuntherapie is de enige behandeling die bij alopecia areata tot een cosmetisch bevredigend resultaat kan leiden

10 De rol van stress bij het ontstaan van veel huidziekten wordt sterk overdreven, de stress is meestal een gevolg van deze huidaandoeningen.

11 De kwalificaties "echte clinicus" of "echte wetenschapper" worden vaak als alibi gebruikt voor het hebben van weinig affiniteit met wetenschap respectievelijk kliniek.

12. Het woord "zalf" in "kwakzalverij" suggereert dat kwakzalvers zich voornamelijk bezighouden met huidziekten.

13 Een zalfkwakker is nog geen kwakzalver.

14. Voor een lage drempel in de gezondheidzorg zijn veel artsen nodig

15. Een volkswagen is ook voor Leidenaren.

16. Paranimfen zijn een levende decoratie (J.M.B.J. van Luyken)

17. Flexibiliteit dient niet te worden verward met chaos.

Nijmegen, 7 december 1994 P M. Steijlen